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U. S. ARMY MEDICAL RESEARCH & NUTRITION LABORATORY

ANNUAL RESEARCH PROGRESS REPORT

1 JULY 1961 TO JUNE 1962

ON

PROJECT 6X60-01-001

INTERNAL MEDICINE

PROJECT 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

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Project No. 6X60-01-001

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BASIC RESEARCH IN LIFE SCIENCES

Project No. 6X99-26-001

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ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 01 - Cardiopulmonary-renal Diseases

Subtask No. 4-3 Microbiological Aspects of Research and
Preventive Medicine Activities in Tuberculosis

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Acid-fast Section, Microbiology Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project Np. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-renal Diseases

Sub-task No. 4-3 Microbiological Aspects of Research and Preventive Medicine Activities in Tuberculosis

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Col L. R. Kuhn, MSC, Lt Col W. C. Morse, MSC, Mary Rothlauf, M. S., Jean Pickering Deter, B. S.

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Tuberculosis laboratory procedures are being revised and simplified, permitting isolation, identification, and drug susceptibility studies to be completed in two to four weeks. Results of bioassays have shown that INH is stable in frozen plasma and serum. The mode of action of isopropyl isoniazid (IPH) seems to be different than that of isoniazid (INH) since IPH is inhibitory to certain INH-resistant tubercle bacilli both in vitro and in vivo. P-amino salicylic acid (PAS) concentrations in serums of patients receiving PAS-C are higher and remain higher in comparison with levels from patients receiving other forms of PAS. Patient acceptance and tolerance of PAS-C is remarkable. Desoxy-ribose nucleic acid (DNA) is being recovered and purified from drug-resistant M. tuberculosis strains. This DNA will be used in resistance transfer studies. Yeast-phase Histoplasma appeared to enhance infection with M. tuberculosis injected concomittantly. Special culture technics have been devised to enhance the growth of tubercle bacilli from surgically resected lung tissue.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-renal Diseases

Sub-task No. 4-3 Microbiological Aspects of Research and Preventive Medicine
Activities in Tuberculosis

Description:

- a. Improvement in methods for isolating and identifying genera and species of Mycobacteria, and for determining drug susceptibilities.
- b. 1. Biologic assays for isoniazid (INH) in the serum of individuals of different racial groups.
2. Stability of INH in frozen plasma and serum.
- c. Studies on the serum concentrations of p-amino salicylic acid (PAS) using a new form of PAS.
- d. An in vitro and in vivo study of the anti-tuberculous activity of chemically pure isopropyl isoniazid (IPH).
- e. The transfer of drug resistance by use of chemically extracted and purified desoxy-ribose nucleic acid (DNA).
- f. The effect of simultaneously injecting M. tuberculosis and yeast phase Histoplasma into mice.
- g. The correlation of histologic changes and bacteriologic findings from tissue sections.

Progress:

- a. The laboratory procedures used by this unit for isolating and identifying mycobacteria are again being modified to include new biochemical methods which will simplify the problems of distinguishing the

"unclassified atypical tubercle bacilli" from M. tuberculosis strains and from the non-pathogenic mycobacteria. A revised edition of this unit's published procedures is being prepared for printing and appropriate distribution.

b. 1. Serum concentrations of isoniazid (INH) are being assayed biologically on all tuberculosis patients admitted to Fitzsimons General Hospital as a guide to the INH dosage which will be required to effect chemotherapeutic concentrations yet not cause certain toxic effects which develop following continued administration of this drug. High serum INH concentrations may lead to two types of toxicity. One is a neural effect which may be delayed or prevented by the simultaneous administration of pyridoxine (B_6). The other is the development of mental aberrations due to elevated blood ammonia levels. Increases in blood ammonia concentrations occur in all individuals taking isoniazid though not all patients develop mental difficulties. (See the Chemistry Division Annual Report by Dr. Z. Z. Ziporin.)

Since the discovery that Japanese metabolize isoniazid to inactive derivatives far more rapidly than Caucasians, serum from tuberculosis patients of other racial groups receiving isoniazid has been, or is being, assayed for isoniazid concentrations. So far, serums from three distinct racial groups in Malaya (Malayans, Tamils and Chinese) have been studied. Results suggest that the Malayans and Tamils inactivate INH slowly (similar to 95% of Caucasians) whereas the results on the Chinese are more consistent with those of the rapidly inactivating Japanese. A study is in progress on the serum of one hundred tuberculous patients from Amman, Jordan. The results of both these studies will be presented for publication in the near future.

2. During the past two years two publications from other laboratories have presented evidence indicating that isoniazid is unstable in frozen plasma and serum. The authors of these publications suggested that their data would render valueless the results of previous work done on plasma and serum isoniazid concentrations. In both these studies chemical assay procedures were used and both authors suggested that the bioassay technic, which measures drug inhibiting activity against multiplying tubercle bacilli, is not sufficiently accurate to detect loss of isoniazid in plasma and serum.

Precise quantitative studies in this division during the past year prove conclusively that isoniazid is stable in frozen plasma and serum for a period up to six months and perhaps longer. These findings were obtained by using a modification of the bioassay technic first described from this laboratory. It is interesting to note that the two workers referred to above, who published on the instability of isoniazid, now agree with our findings. (See publications.)

c. Studies have continued on the serum concentrations of p-amino salicylic acid (PAS) achieved in tuberculosis patients receiving a new form of PAS which is trade-marked PAS-C (PAScorbate). In the last annual report PAS-C serum concentrations were compared with those obtained with other forms of PAS. The findings indicated that six grams daily of PAS-C achieved serum concentrations equivalent to those obtained with 12 grams of other forms of PAS tested. At that time PAS-C was considered to be a complex of p-amino salicylic acid and ascorbic acid. Considerable detailed investigation on PAS-C in this

unit (See Chemistry Division, Dr. Z. Z. Ziporin.) has now shown that PAS-C is not a complex of PAS with Vitamin C but rather a highly purified form of PAS acid. It has not yet been determined why significantly higher blood concentrations are obtained with this product as compared to other forms of PAS. Tolerance and acceptance of PAS-C by patients continues to be excellent. Studies on the unique biological activity and biochemistry of PAS-C will continue during the coming year.

d. Preliminary in vitro and in vivo studies indicated that the isopropyl derivative of isoniazid (IPH) was inhibitory to certain isoniazid-resistant strains of mammalian tubercle bacilli. These results suggested that the mode of action of iproniazid was different than that of isoniazid (INH). Further studies have revealed that IPH is inhibitory in vitro and in vivo (white mice) only for isoniazid-resistant M. tuberculosis strains which have lost the ability to synthesize endogenous catalase. The exact significance of these observations has not yet been characterized. In collaboration with Dr. Oscar Reiss, Chief Biochemist, Webb Institute for Medical Research, University of Colorado Medical School, further investigations are to be made.

e. Extensive studies were made on the extraction, purification, and identification of desoxy ribose nucleic acid (DNA) obtained from INH-resistant (INH-R) strains of tubercle bacilli. This DNA will be used in attempts to transfer INH resistance to drug-susceptible tubercle bacilli. Considerable difficulty was experienced because the cell destruction technique ruptured relatively few bacilli.

Recovered substances exhibited spectrophotometric absorption characteristics consistent with DNA but the quantitative yield was extremely low. Also, it was believed possible that the spectrophotometric absorption tests were indicating DNA residues and not the unaltered material necessary for successful transfer of resistance. A new Shandon-Hughes cell press has been installed and the studies on recovering DNA will continue.

f. White mice were injected intravenously with M. tuberculosis strain H37Rv and yeast-phase Histoplasma capsulatum simultaneously and the extent of infection at 5 to 8 weeks determined by measuring density of the mouse lungs and comparing with similar measurements on mice injected with each agent alone. Lungs from mice receiving both agents showed a significant increase in density over lungs from mice given only M. tuberculosis. Those given yeast-phase Histoplasma were no different than the normal (i.e. uninjected) controls. At autopsy, only M. tuberculosis could be recovered by culturing, not Histoplasma. Histological studies are in progress and further experiments are planned in the hope of explaining why unsuccessful infection with yeast-phase Histoplasma should enhance infection with M. tuberculosis.

g. This is a cooperative study to assist the Pathology Service, Fitzsimons General Hospital in correlating the observed histologic changes with bacteriologic findings by studying the growth of tubercle bacilli in sections of surgically removed lung tissue cut at 6 - 12 microns. Serial sections are prepared and treated with special stains while alternating sections are submitted for culture.

Preliminary studies were made using lungs of mice experimentally challenged intravenously or intraperitoneally with virulent tubercle bacilli. Two culture technics were used:

(1) Cut sections were placed on the surface of an Oleic acid-albumin agar medium (7H10) and incubated at 36°C, then examined at weekly intervals.

(2) Cut sections were placed on sterile glass microscope slide or cover slips and immersed in a liquid oleic acid-albumin medium, then incubated at 36°C and examined at two day intervals macroscopically and by low power microscopy.

Preliminary results indicate that the culture technic using sections affixed to glass slides or cover slips immersed in liquid medium is superior to the use of a solid medium. Growth of tubercle bacilli was observed in as short a time as 4 days of incubation using this experimental approach.

Studies applying these technics to human lung tissue taken at surgery are now underway.

Summary and Conclusions:

a. Tuberculosis laboratory procedures are being revised and simplified, permitting isolation, identification, and drug susceptibility studies to be completed in two to four weeks.

b. Results of microbiologic assays for serum isoniazid (INH) are being used to control drug dosage and to characterize different racial groups into rapid or slow inactivators of this drug. Results of bioassays have shown that INH is stable in frozen plasma and serum.

c. P-amino salicylic acid (PAS) concentrations in serums of patients receiving PAS-C are higher and remain higher in comparison with levels from patients receiving other forms of PAS. Patient acceptance and tolerance of PAS-C is remarkable.

d. The mode of action of isopropyl isoniazid (IPH) seems to be different than that of isoniazid (INH) since IPH is inhibitory to certain INH-resistant tubercle bacilli both in vitro and in vivo.

e. Desoxy-ribose nucleic acid (DNA) is being recovered and purified from drug-resistant M. tuberculosis strains. This DNA will be used in resistance transfer studies.

f. Although apparently failing to survive in intravenously injected mice, yeast-phase Histoplasma appears to enhance infection with M. tuberculosis injected concomittantly.

g. Special culture technics have been devised to enhance the growth of tubercle bacilli from surgically resected lung tissue. Sections of lung tissue, (6 - 12 microns thick), from experimentally infected mice, are placed on glass cover slips or slides and immersed in an oleic acid-albumin liquid medium for incubation. These have given positive results in 4 to 8 days. Studies are being continued.

List of Publications:

Stability of Isoniazid in Plasma and Serum, W. C. Morse, J. M. Lacerte, N. J. Pickering and L. R. Kuhn. Transactions of the 21st VA-Armed Forces Conference on the Chemotherapy of Tuberculosis, VA Central Office, Washington 25, D. C. American Review of Respiratory Diseases. Both in press to be published in 1962.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 01 - Cardiopulmonary-renal Diseases

Sub-task No. 4-4 Microbiological Aspects of Research and
Preventive Medicine Activities in Mycotic Infections

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Mycology Section, Microbiology Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-renal Diseases

Sub-task No. 4-4 Microbiological Aspects of Research and Preventive Medicine
Activities in Mycotic Infections

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General
Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Col L. R. Kuhn, Sp 7 Leroy Nolte, and Erma Sproat, B. A.

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Infection of mice with intravenously injected
Histoplasma capsulatum in the yeast phase was un-
successful. Yeast phase Histoplasma appears to grow
well at 40°C. A review of three years' experience
with serology in pulmonary fungus diseases has been
completed.

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BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-renal Diseases

Sub-task No. 4-4 Microbiological Aspects of Research and Preventive Medicine
Activities in Mycotic Infections

Description:

- a. Attempts to produce experimental infection with yeast-phase Histoplasma capsulatum.
- b. Growth of yeast-phase Histoplasma at higher than human body temperatures.
- c. Cultural and serological tests in patients with pulmonary disease.

Progress:

- a. Laboratory acquired infections represent a hazard to personnel handling the mycelial-phases of Histoplasma and Coccidioides. Use of the yeast-phase of Histoplasma for injecting mice intravenously should reduce this hazard and increase the use of experimentally infected animals for therapeutic and other studies. Accordingly, Histoplasma capsulatum was successfully maintained in the yeast-phase for injecting mice. Although the in vitro yeast-phase would seem to be more closely related to the tissue-phase of this fungus, we have not succeeded in producing progressive infection in mice injected intravenously with yeast-phase cultures. It may be that the initial resistance to the body defense mechanisms is lacking in such cultures. Also, when yeast-phase cells were injected simultaneously with M. tuberculosis, Histoplasma could not be cultured from tissues five to eight weeks after infection. However, infection with M. tuberculosis appeared to have been

enhanced in these animals (see report from the Tuberculosis Section). Last year, we reported unsuccessful attempts to infect rabbits with yeast-phase *Histoplasma*. However, since these animals have a considerably higher body temperature, (39-40°C), than mice, (37.5°C), this was thought to be a contributing factor.

b. Some years ago, the senior investigator found that temperatures slightly in excess of normal human body temperature were unfavorable to the growth of *Cryptococcus neoformans* in vitro and in vivo. As indicated in (a), it was considered that *Histoplasma capsulatum* might show a similar deleterious effect from such an environment and that this might then be useful as an adjunct to chemotherapy. Although only rough quantitative estimates were made, yeast-phase *Histoplasma*, unlike *Cryptococcus*, appears to grow equally well at human normal and fever temperatures.

c. Cultural and serological support in mycology for the Pulmonary Disease Service of Fitzsimons General Hospital has been maintained. Captain May, M. C., of the Pulmonary Disease Service, has just completed a study of the serological findings from our records and interpreted these in the light of clinical progress of patients. A paper prepared by Captain May from this study won for him the prize given annually by the Fitzsimons General Hospital for papers presented by residents.

Summary and Conclusions:

Infection of mice with intravenously injected *Histoplasma capsulatum* in the yeast-phase was unsuccessful. Yeast-phase *Histoplasma* appears to grow well at 40°C. A review of three years' experience with serology in pulmonary fungus diseases has been completed.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 01 - Cardiopulmonary-renal Diseases

Sub-task No. 4-5 Microbiological Support for Preventive Medicine
and Clinical Research in Infections other than
Tuberculosis and the Mycoses

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bacteriology Section, Microbiology Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-renal Diseases

Sub-task No. 4-5 Microbiological Support for Preventive Medicine and Clinical Research in Infections other than Tuberculosis and the Mycoses

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Col L. R. Kuhn, O. L. Weiser, Ann Hinds, Joe S. Emerson

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Unclassified

A qualitative and quantitative laboratory and clinical study of pyelonephritis was begun in order to define adequate treatment and incidence of relapse. A study of adeno and influenza viruses in three population groups was begun. A study of staphylococcus carriers indicates that there is an unusually low nasal carrier rate for coagulase-producing staphylococci in this area. However, "hospital types" appear to be increasingly common in non-hospital personnel. Furacin spray and bacitracin ointment did not remove coagulase-positive staphylococci from the external nares of carriers.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-renal Disease

Sub-task No. 4-5 Microbiological Support for Preventive Medicine and Clinical Research in Infections other than Tuberculosis and the Mycoses

Description:

a. A study utilizing precise bacteriologic methods for quantitative urine cultures and an evaluation of therapeutic measures.

b. A study of viruses associated with chest diseases, both chronic and acute.

c. A study of the pathogenic *Staphylococcus* carrier state in a hospital population, with emphasis on methods of treating carriers.

Progress:

a. 1. Pyelonephritis is an important but commonly overlooked disease. Recent studies have shown that a quantitative bacteriological study of patient's urine is a valuable measure of the extent of infection and therapeutic progress. The Department of Medicine, Fitzsimons General Hospital, requested this division to collaborate with the Pediatrics Service in a study involving precise bacteriologic methods for quantitative urine cultures. The results of these studies will be used to define an adequate duration of treatment for complicated and uncomplicated urinary tract infections, and to determine the incidence of clinical and bacteriologic relapse following two therapeutic regimens.

2. The project was started by evaluating methods of collection and methods of accurately counting and identifying organisms found in patients with bacteriuria. Collection methods used at Fitzsimons General Hospital were found to be satisfactory if the urines were

delivered to the laboratory promptly. Delay in delivery was accompanied by decreasing numbers of viable organisms more frequently than the expected increase. Methods of bacteriologic evaluation included gram stain, pour plates, and glass-rod streak plates from undiluted through the 10^5 dilution of urine. All plates were prepared in duplicate. The streak plates gave somewhat higher counts than pour plates, but had the disadvantage of being uncountable in the presence of spreading and swarming. Only a minimum count of 10,000 organisms per ml of urine is considered positive for urinary tract bacteriuria. Antibiotic sensitivities were determined and selective media were employed for rapid identification. Interpretation of results will follow clinical evaluations.

3. To date, 400 urines have been cultured by the methods outlined. Sixty of the 400 urines cultured had counts of 10,000 or more bacteria per ml. The organism most frequently cultured was E. coli with nineteen cultures positive. Pseudomonas was cultured nine times, Proteus and Aerobacter four times each. Other organisms cultured were Paracolon sp., Staphylococcus and Enterococcus. Mixed infections were not uncommon. Twenty of the significant positive cultures were E. coli and other Proteus sp., Pseudomonas sp. or Aerobacter.

4. A study of fifty patients is projected. To date no patient has completed the six-month follow-up period. Five patients have been studied through four months.

b. 1. The Pulmonary Disease Service of Fitzsimons General Hospital requested direct support in studies of viruses associated with chest diseases,

both chronic and acute. Facilities for these studies were made available in August 1961, the viruses studied to be limited, at present, to the adeno and influenza groups.

Collection of material from patients was started in September 1961 from three different groups:

i. In-patients with acute respiratory disease (ARD) hospitalized in the non-tuberculosis wards at Fitzsimons General Hospital. Patients for study were selected by the Ward Doctor.

ii. Patients in the upper respiratory infection (URI) ward at Fort Carson Army Hospital.

iii. Patients from Lowry Air Force Base. Specimens from this group were collected and forwarded to the Department of Medicine, Colorado Medical School.

2. Throat washings and acute blood samples have been collected on 297 patients. Convalescent blood has been obtained on approximately 35% of these patients and serologic tests using influenza A and B and adenovirus group antigens have been completed. Significant diagnostic titres to adenovirus group were demonstrated in 59% of the sera tested. Thirteen percent were positive for influenza B. Only one serum showed a significant increase in titre to the influenza A antigen. Throat washings from patients with significant complement-fixing titres were passed through 10 - 12 day hen's eggs. Influenza B virus has been isolated from three of the throat washings to date.

3. Throat washings from all cases are being passed in cell lines (Hela, MK, HE_p²), Cytopathogenic effects have been observed

in approximately 30% of the cells inoculated. Adenovirus type-specific antisera is being prepared to determine which type was present this past winter.

4. All sera collected were tested for the presence of cold-agglutinins and significant titres were obtained in 21% of the tests.

No evidence for the publicized influenza B epidemic was found.

However, some cases of influenza B were noted at Fort Carson between 17 January and 20 February. During this period 14% of sera tested had significant rises in complement-fixing antibodies. This was not seen in the Fitzsimons or Lowry groups and was not reported in the civilian population of Colorado Springs.

5. Studies on the material collected this past season is continuing.

c. 1. With the consent of the Fitzsimons General Hospital Research Committee and the Departments of Surgery and Medicine, a study of pathogenic staphylococci in the Post population and in the hospital was begun. Several years ago, a study of the nasal carrier rate for coagulase-positive staphylococci in the hospital population had indicated a much lower rate than is usually reported, i.e. 20-30% compared to 40-70%. During the past year, nasal cultures were taken from all personnel and dependents who are given annual chest X-rays and tuberculin tests. A much lower carrier rate than is usually reported for an urban population again was noted. On the other hand, among the fewer carriers there was a higher percentage of penicillin-resistant staphylococci and the so-called "hospital epidemic strains" showing susceptibility to phages 80-81 than was expected. It is possible that this reflects an increasingly commoner carrier state with penicillin-resistant and "hospital phage types" among non-hospital groups.

2. The external nares of all newborn infants and their mothers are being cultured prior to their release from the hospital. These are again cultured six weeks later when they return to the Pediatrics Service. Interesting findings are already apparent; namely, that not only do some acquire "hospital types" following hospitalization, but that some mothers now are carriers of these types prior to hospitalization. The study will soon be extended to patients on arrival for surgery and before discharge.

3. In collaboration with the Ear-Nose-Throat Service, attempts were made to eliminate the nasal carrier state by means of furacin nasal spray and bacitracin ointment applied to the external nares in some twenty-five persons who had repeatedly positive cultures of coagulase-positive staphylococci. Although a few persons in each group appeared to lose their coagulase-positive staphylococci, the great majority of carrier states persisted despite treatment. Other substances also will be tried.

4. Commercial availability of standard bacteriophages and new techniques have simplified the phage-typing procedure and greatly increased the numbers of staphylococci which can be typed per unit time.

Summary and Conclusions:

a. A qualitative and quantitative laboratory and clinical study of pyelonephritis was begun in order to define adequate treatment and incidence of relapse. Fifty patients will be studied. To date, 400 specimens have been examined, but only five patients followed through four months.

b. Facilities for respiratory virus studies were acquired and personnel trained. A study of adeno and influenza viruses in three groups was begun.

c. 1. Fitzsimons Post personnel show an unusually low nasal carrier rate for coagulase-positive staphylococci, but many carriers now harbor penicillin-resistant and "hospital types", probably reflecting an increase in carriers of this description.

2. Some newborn infants and mothers appear to acquire "hospital types" during hospitalization, but some mothers harbor them prior to hospitalization.

3. Furacin nasal spray and bacitracin ointment failed to remove coagulase-positive staphylococci from the external nares of carriers.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 01

Cardiopulmonary-Renal Diseases

Sub-task No. 5-12 A Study of the Various Plastic Materials
In Arterial Grafting

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

J. Harold Harrison, M.D.
and
Robert M. Nims, Lt. Col., VC

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-Renal Diseases

Sub-task No. 5-12 A Study of the Various Plastic Materials
In Arterial Grafting

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: J. Harold Harrison and Robert M. Nims

RCS MEDDH-288

Unclassified

This project is inactive except for observation of the long-term survival animals. There are seven survivors, ranging from 6 to $7\frac{1}{2}$ years with various prosthetic materials replacing the thoracic aorta.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-Renal Diseases

Sub-task No. 5-12 A Study of the Various Plastic Materials
In Arterial Grafting

Description:

A study of the efficiency of various synthetic materials for use as large vessel prostheses.

Progress:

During this fiscal year one animal expired. It had survived 5-3/4 years with a Teflon fabric graft. Death was due to embolism of the mesenteric vessels with resultant necrosis of the small bowel. There was no evidence of erosion of the Teflon prosthesis; however, there was a mature thrombus attached to the inner surface of the prosthesis. This was probably the origin of the emboli.

There are seven remaining dogs containing prostheses of various materials, from 6 to 7 1/2 years of age. At present these animals are clinically normal.

Summary and Conclusions:

None. Long-term survival studies will continue on the remaining animals.

List of Publications:

None during the current year.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 01

Cardiopulmonary-Renal Diseases

Sub-task No. 5-28 Nutritional Factors Influencing the
Cardiovascular System

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 February 1962 - 30 June 1962

James A. Vogel, 1/Lt., MSC
and
Mavis S. Baldwin, SP4

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-Renal Diseases

Sub-task No. 5-28 Nutritional Factors Influencing the Cardio-vascular System

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 February 1962 - 30 June 1962

Authors: James A. Vogel and Mavis S. Baldwin

RCS MEDDH-288

Unclassified

In addition to atherosclerosis, much needs to be known concerning the interaction of nutrition and circulatory physiology. Experiments have been initiated to study the interrelationships of specific nutrients and states of nutrition to this system by means of a previous-starvation technique. Methodology development for this work is still in progress.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-Renal Diseases

Sub-task No. 5-28 Nutritional Factors Influencing the Cardio-vascular System

Description:

Such clinical problems as essential hypertension and atherosclerosis have pointed out the necessity of a more thorough understanding of the basic interrelations between nutrition and the cardiovascular system. To this end, experiments have been planned using rabbits to study the neural and endocrine pathways of nutritional influences on the circulatory system. Initial trials involve the effects of starvation. Later experiments will involve food deprivation to achieve a base line from which the effects of individual nutrients may be studied. Cardiac output, blood pressure, blood volume and related parameters are being studied.

Progress:

In order to measure cardiac output and vascular resistance in unanesthetized rabbits over a period of weeks, it was found necessary to implant chronically Silastic catheters (Dow-Corning) near the heart via the common carotid and jugular vessels. It was found that the functional use of the arterial catheter does not ordinarily last beyond one week, seemingly due to movement of the catheter tip rather than thrombosis. To avoid this difficulty, an external body counting isotope technique has been tested which makes it unnecessary to use

5-28, Progress (Cont'd)

an arterial catheter. Venous catheterization is desirable for injection of the tracer. It has also become apparent that the determination of blood pressure by electronic sphygmomanometry involves some difficulties. For these reasons it has been decided to use dogs for these experiments. Cardiac output by dye dilution and blood pressure by sphygmomanometry in the conscious dog have been developed. Comparisons of the external counting method to the dye method for cardiac output are now being made and it is anticipated that the former technique will be employed.

Summary and Conclusions:

While developing cardiovascular procedures in the rabbit for these experiments, a number of inherent disadvantages have been found with this animal. For this reason, techniques are now being developed by which a complete analysis of the circulatory system can be made in the unanesthetized dog. This includes cardiac output, vascular resistance and volume by isotope-dilution techniques and arterial blood pressure by electronic sphygmomanometry.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 01

Cardiopulmonary-Renal Diseases

Sub-task No. 7-10 Study in Replacement of Thoracic
and Cervical Trachea

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

Elmore M. Aronstam, Lt. Col., MC
and
Robert M. Nims, Lt. Col., VC

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-Renal Diseases

Sub-task No. 7-10 Study in Replacement of Thoracic and
Cervical Trachea

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Elmore M. Aronstam and Robert M. Nims

RCS MEDDH-288

Unclassified

In a continuing study of tracheal prostheses, five series of animals, totaling twenty-eight dogs, have been conducted. Three of these have been failures, the other two series are still being followed. One series is not an acceptable result but indicates it may show increased longevity. The final series is too early to predict results. The best longevity to date has been fifteen months.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 01 Cardiopulmonary-Renal Diseases

Sub-task No. 7-10 Study in Replacement of Thoracic and Cervical Trachea

Description:

Evaluation of various prosthetic materials and various methods of fixation of segmental tracheal prostheses in an attempt to develop methods and materials for tracheal replacement in patients with tracheal stenosis, deformity, neoplasia or trauma.

Progress:

During the current fiscal year five series of animals have been initiated and are in various phases of study. One series (#10) was reported during the previous year but was still being observed.

Series 10: These prostheses had an inner and outer surface of Dacron cloth with a Silastic rubber core containing 10/1000 inch thick rings of stainless steel 1.5 to 2 mm wide to simulate tracheal rings. Overall thickness was approximately 1 mm.

Fixation was with 3-0 Dacron sutures. Buttresses were replaced by a ring of Silastic covered Dacron suture which was so sized as to fit snugly around the trachea containing the prosthesis. This assured a close fit between the trachea and prosthesis.

7-10, Progress, Cont'd

In this series the trachea was carefully dissected free from surrounding tissues only enough to permit proper fixation in an attempt to create minimal disturbance to vasculature. Also, four of the mattress sutures on each suture line were tied together with their mates on the opposite suture line to minimize longitudinal stress on the suture line.

Of five animals in this series, one died at $5\frac{1}{2}$ months with large granulations and stenosis. Two survived to 10 months, one to 13 months. The final animal is alive at 15 months. There is some loosening of sutures and slight surface infection, but the site appears quite well stabilized.

Series 11: Four dogs received a solid Terpolymer prosthesis fabricated by the Army Prosthetic Research Laboratory, Walter Reed Army Medical Center. Two were inserted using no sutures, fixation being achieved with Eastman 910 adhesive (methyl-2-cyanoacrylate). One dog died at surgery from anesthesia. The other succumbed at $3\frac{1}{2}$ months, two weeks after the loose prosthesis was removed, allowing the trachea to stenose.

Two prostheses were inserted using the Eastman adhesive with sutures. One survived 10 months, death being due to stenosis after the loose prosthesis was expelled by the animal. The other is alive at 7 months, shows moderate granulations and slight infection. This series is considered a failure.

7-10, Progress, Cont'd

Series 12: This series used the same prostheses and technique as Series 10, except the stainless steel support was a spiral instead of rings, and Eastman 910 adhesive was utilized along with sutures.

Five dogs were utilized. Four died within 4 months due to failure of the prostheses. This was probably due to over sterilization, which caused the layers of the prosthesis to separate with resultant asphyxia. The other animal is alive at 11 months. However, the prosthesis is becoming loose, there is slight to moderate granulation and infection.

Series 13: The prosthesis used was of solid Terpolymer, as in Series 11, to which a Dacron and foamed Terpolymer cuff had been affixed at each end to allow tissue growth to form a bond between the prosthesis and trachea. The prostheses were secured with Eastman 910 adhesive and sutures. Four dogs were utilized.

One dog died from postoperative pneumothorax. Two died (4 and 7 weeks) from the prosthesis loosening and moving with resultant stenosis at the graft site. The last animal is alive at 7 months but has severe dyspnea from stenosis which has reduced the diameter of the tracheal lumen by greater than 50%.

Series 14: This series used a prosthesis identical to Series 12. Eastman 910 adhesive was used, however, only four mattress sutures were placed in each suture line. The matching

7-10, Progress, Cont'd

sutures from each suture line were tied together spanning the outer longitudinal surface of the prosthesis to reduce the longitudinal stress on the suture lines.

There were 9 animals in this series. There were 3 early deaths; one each at 2 and 7 weeks from pneumonia and one dog was inadvertently euthanatized.

Three others, alive at 2, 3 and 4 months, are failures due to fragmentation of the prosthesis, probably due to repeated sterilization of the prosthesis with resultant separation of the layers.

The remaining three are alive at 4, 6 and 7 months. One (4 months) has loosened and become infected. One (6 months) shows slight granulations, no evidence of infection and is considered fair. The other (7 months) is infected, has some pus and debris but shows no signs of immediate failure.

Series 15: This is the same basic prosthesis as Series 12 and 14, using four mattress sutures on each suture row and tied together longitudinally as in Series 14. The mattress sutures were tied over an encircling Silastic coated Dacron ring which served as a buttress. The prosthesis was placed deeper into the trachea, so that the sutures were placed between the second and third intercartilagenous ring. The cartilagenous rings overlying the prosthesis were brought snug about it by a mattress suture connecting the free cartilage ends.

7-10, Progress, Cont'd

Two of the prostheses were glued using the Eastman 910 adhesive to supplement the sutures. One dog died at one month from anesthesia during bronchoscopy. The prosthesis was satisfactory. The other dog is alive at 4 months, has evidence of infection but no granulations. The cranial suture line is quite loose.

Four prostheses were placed without use of the adhesive. At two months following surgery all appear good but more time is needed for final evaluation.

Summary and Conclusions:

This past year, five series of dogs have been utilized to continue our study of tracheal prosthetic materials and methods of fixation. Monthly bronchoscopic examinations are performed to continually evaluate the progress of the dogs.

Thus far, no satisfactory solution to a tracheal prosthesis has been found. Experience gained indicates:

(a) The Eastman 910 adhesive is of no advantage for the Terpolymer or Dacron and Silastic prostheses. It does not permanently bond the prosthesis to the trachea.

(b) Although the prosthesis should be as thin as practicable, it should have enough rigidity to prevent crimping and pleating which creates dead space between the prosthesis and trachea. When this occurs, infection and granulation seems inevitable.

7-10, Summary and Conclusions, Cont'd

(c) The use of eight mattress sutures on each suture row appears preferable to four. Four sutures do not give adequate fixation.

Further study will investigate new materials as they become available. Also, better methods of fixation and simpler techniques will be attempted.

List of Publications:

1. Aronstam, Elmore M., Nims, Robert M., Winn, D. F., Jr.:
Studies in Segmental Replacement of the Thoracic Trachea.
J. Surg. Research 1:108, 1961

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 01 - Cardiopulmonary-renal Diseases

Sub-task No. 8-1 A Study of the Application of Electronic Data
Processing in Medical Research and Clinical Practice:
The Development of A Logic System for Classifying
Pulmonary Disability

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Automatic Data Processing Systems Section

1 July 1961 - 30 June 1962

Lt. Col. J. C. Syner, M.C.
A. B. Ruffner
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ABSTRACT

Project No. 6X60-01-001

Task No. 01 - Cardiopulmonary-renal Diseases

**Sub-task No. 8-1 A Study of the Application of Electronic Data
Processing in Medical Research and Clinical Practice:
The Development of A Logic System for Classifying
Pulmonary Disability**

**U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado**

1 July 1961 - 30 June 1962

Authors: Lt. Col. J. C. Syner, A. B. Ruffner, J. M. Deshotels

RCS MEDDH-288

Unclassified

A computer controlled system utilizing an RCA 301 Electronic Computer, 20K memory capacity, paper tape input, output, monitor printing device, and Data Record File is being developed. A Flexowriter-Selectadata complex will be utilized for generating punched paper tape and printing some contents of the computer High Speed Memory dumped on paper tape.

A computer simulated model of disability evaluation in diseases of the chest is being programmed. This model utilizes some 476 individual items of administrative, medical, and laboratory data per patient from the patient's medical record. Many of these items recur in multiple sequences for each patient. The object is to develop a logic system for deriving a clinio-patho-physiological classification of pulmonary insufficiency equated in degrees of disability.

In addition to the defined principal problem the data and computer programming permits actions of model simulation, process control, information storage, analyses and synthesis of probability tables translating, indexing and compiling in various medical areas.

BODY OF REPORT

Project No. 6X60-01-001

Task No. 01 - Cardiopulmonary-renal Diseases

Sub-task No. 8-1 A Study of the Application of Electronic Data Processing in Medical Research and Clinical Practice:
The Development of A Logic System for Classifying
Pulmonary Disability

Description:

This research effort has been conceived and organized to study the utilization of electronic data processing arts and sciences in clinical medicine and medical research as practiced in the U. S. Army Medical Corps.

As an initiating approach to exploring this broad research area of high generality a particular problem of low generality has been conceived, designed and organized for operation in the environment of a computer-controlled center. The particular problem is the development of a logic system for deriving a clinico-patho-physiologic classification of pulmonary insufficiency equated to the degree of disability for military service. In military medicine there is a need for a standardized system for objectively evaluating degrees of physical disability and fitness for use in military medical installations on a world wide basis.

The problem is organized around a system designed to provide computer programmed simulation of the disability evaluation problem. The computer will be programmed, through appropriate data and instructions, to behave as if it were the actual physical

system. To produce a realistic state of simulation the processes of the system, including both physical and mental actions, must be formulated, through mathematical techniques and symbolic logic, into a model. In this way a study can be made of the concepts and hypotheses as to how the system works, its resulting decisions of strategies and values, and to discriminatory level of these decisions relative to alternate lines of action.

The data pool required to process such a logic system embraces nearly the entire patient's medical record. Table No. 1, Record File Structure, summarizes the composition of information utilized in this problem. In its current form, in our system, the individual patient-file contains approximately 476 separate items of information per patient. These items cover the gamut of administrative, medical, and laboratory data required to evaluate a problem of pulmonary insufficiency in disability profiling. As previously stated, it is currently estimated that some 10,800,000 characters of information will be functioning in the simulated computer model at the peak of its activity. Such a model is designed to function, when properly verified, on a daily basis in line medical evaluation problems in a computer controlled center.

In addition to the patient file, clinical and laboratory data on approximately 1000 normal subjects, studied at the time of annual physical examination, are included in the system. These data will function as comparative and correlative forces in our system. The system provides for accumulating continuum of data on normal subjects in monitoring annual physical examinations in military personnel.

A computer program library of some 100 varied programs (principal routines and subroutines) will be required in this study. They will consist of the following broad types of programs:

1. Classical Statistical Routines, such as Standard deviations, Cross products, Correlation coefficients, Factor analyses, Analyses of Variance, etc.

2. Logic-Housekeeping Routines, such as sort, file, extraction, sequence, inputs, data conversions, file updating, quality controls, print out, etc.

3. Symbolic Logic Routines, such as diagnostic routines to develop sets and classes from the "soft" qualitative information of the medical arts and sciences.

4. Classification Program so as to order the data into its ultimate image of control.

In meeting these requirements the computer controlled center will be programmed to function in other medical areas of analyses and syntheses, simulation, data compilation, table construction, information storage, and education.

Progress:

During this period of preparations for on site installations of the RCA 301 Electronic Computer the primary efforts have been in developing a system to handle input requirements (collection, machine translation and storage), analyses of data, and output requirements. The major emphases has been in the development of

data-input formations from collecting information from the patient's medical and health records.

Formal education and training has been received in flow charting, computer logic, computer programming, scientific interpreter and flexowriter-selectadata operations and programming. Several consulting visits with members of the RCA technical and educational staffs, including Dr. Carl Hammer, Chief, Scientific Division, have been conducted. Some of these visits have extended over 3-4 day periods.

I. Input.

The operation for conversion of information from manual copy to machine codes stored on magnetic tapes has been completed. All of our 80 column cards have been translated to magnetic tapes. A Flexowriter-Selectadata complex was utilized to generate punched paper tape in RCA 501 Electronic Computer Language. It was then computer programmed for sorting and filing into specific categories in the RCA 501 computer. It is now computer programmed for conversion from RCA 501 language into RCA 301 language so that it can be transferred to RCA 301 paper tape and then stored into our Master Files in the RCA 301 magnetic Data Record Files.

The data from this source was edited for quality assurance and found to be extremely accurate. The ratio of errors was determined to be 1 out of every 20,000 characters of information. The majority of the few errors discovered would have been corrected by our existing computer program edit routines.

The amount of information involved can be appreciated by reference to Table No. 1, Record File Structure, which summarizes the organization and contents of our Record File Structure. At this time information has been collected, machine coded, stored and filed on magnetic tapes in the categories listed as: O. Code-Name, Code Index; 1. Code-Name, Name; G. Allergy, Inhalants and Scratch Tests; H. Allergy Mixes, Scratch and Intradermal Tests; I. Respiratory Disease Questionnaires; J. Smoking History; K. Spirometry; P. Lung Volumes; and R. Treadmill Studies. Information in all of the other categories making up the Record File Structure have been collected and are ready for punching on to paper tape upon delivery of our Flexowriter-Selectadata Complex. The Flexowriter-Selectadata equipment was scheduled for delivery on 1 July but has not yet arrived from the manufacturer.

By computation we now have approximately 2,000,000 characters of information in our system. We have programmed to have 4,500,000 characters by the end of 1962, and 10,800,000 characters by the end of 1963.

In addition to the data already machine coded, sequenced, filed and categorized in specific format on magnetic tapes our system has produced in continuum an amount of clinical, physiological and laboratory information manual copies ready for transcription on to paper tape in machine code for computer input. The current totals of this information is approximately 75,000 characters. Our systems provides for a continuum in the collection of this data. In the near future we hope to perform information

collection and computer input functions on a current, daily, basis.

II. Program Library.

Medical programs, statistical programs and logic-housekeeping programs have been designed and written. The medical programs currently completed or in writing include computation of spirometry values from raw data inputs, a table of frequency-distribution of past and present illness in our patient and normal subject populations, logic programs covering the following disease states: bronchitis obstructive, bronchitis non-obstructive, bronchial asthma, obstructive emphysema, and pulmonary hyperinflation.

Logic-housekeeping routines include programs converting data from paper tape source to High Speed Memory and storage in appropriate Data Record File Locations; extracting data from the Master File and assembling into desired population nodes for study and analyses; producing alpha print outs of descriptive diagnostic terms and logic for patient discrimination.

Systems planning has been developed to accommodate, by intrinsic computer programming, the need for updating and modifying Record File Structure as new data is entered, unwanted data is eliminated and quality-control processing occurs.

The majority of the classical statistical routines required over the first year of operation will be obtained from the RCA Users' Library and will consist of: Floating Decimal Arithmetic

Subroutines, Square Root and Transcendental Subroutines, Linear Equations and Matrix Inversion Programs, Multiple Regression and Correlation Programs, Least Squares Polynomial Fit Program, and Analyses of Variance Programs. These routines provide other desired arithmetic and statistical outputs in the process of computing the principal result. Certain portions of these library programs will need rewriting in order to be compatible with our equipment configuration.

III. Systems Progress.

Systematic review of the processing requirements involving the analyses of the volume and types of data to be processed were initiated. Continual exploration of present data processing methods, alternative possibilities, and benefits which would be realized through computer processing are in existence. Detailed studies were made of every related job, paper, document, and processing operation of the essential elements of information in the current processing system. Consideration of opportunities for generating other needed information or eliminating present steps, processes and information manipulation were also performed.

A study is under way relative to organizational, functional and projected resource requirements of a computer center complex in a medical and scientific environment.

IV. Equipment Selection and Site Preparation.

Studies were made of on site computer installations in the local area to evaluate and analyze the best systems and equipment

in determining the peripheral systems necessary to optimize the needs of our project.

Consultations with manufacturer representatives, attendance of seminars, and conferences with users were also accomplished as an aid to design and selection.

Coordination with area Engineers, local Engineers, RCA Engineers, Purchasing and Contracting personnel and potential contractors was conducted in planning for our computer center.

The contract for site construction was given final award on 9 July 1962 and construction personnel started working the following day. Construction completion date is 90 days after start of work, however, it is expected that the computer could be received for check-out, site test and debugging by computer engineers no later than 1 October 1962.

V. Outputs.

Computer produced listings of all machine converted data, in specific sequences, was accomplished. These listings are indispensable for the inventory of the file composition, appraising validity of data and planning for current and projected actions on the file structure.

Summary and Conclusions:

Experience to date substantiates that this problem is computer oriented and that an excellent state of readiness has been achieved

for on site computer utilization. The essentiality of an on site computer facility to process this problem has been further strengthened by the difficulties over the past year in attempting to rent computer time from other users.

It has been further demonstrated that in-house personnel must acquire and ever strengthen their own capabilities to design, organize, program and operate whatever type of computing center is required for their special areas. You can not depend upon others to do it for you.

The overwhelming importance for developing a solid, intelligent, stable system for operating the computing center has emerged as one of our most vital experiences. The computer controlled center must be organized in detail with great precision.

Our experience to date indicated that additional personnel will be required in the near future. For planning purposes we recommend that the following personnel be programmed for:

1. Flexowriter Operator. GS-4 level.
2. Computer Console Operator. GS-4 level.
3. Two Computer Programmers. GS-9.

The scientific products which will be generated from the computer control center will commence to take form once computer installation is accomplished and the existing system of data collection, machine language conversion, data storage, data retrieval and Record File manipulations are adequately tested and debugged. Once this is accomplished it is planned that during

the first year of on site computer processing major efforts will be devoted to validation and analysis of the numerical information so as to achieve our first approximation of data ordering for patient classification. Time sharing of this operation with the diagnostic programs in symbolic logic and Boolean functions will be accomplished.

List of Publications:

Current report year: None

Past publications:

1. Kory, Ross, C., Callahan, Robert, Boren, Hollis G., and Syner, James C., The Veterans Administration Army Cooperative Study of Pulmonary Function. Am. Jr. Med. 30:243-258, Jan. 1961.

2. Syner, James C., Ruffner, Albert, and Murray, Sarah. Machine Processing of Measurements In Respiratory Gas Exchange and Energy Expenditure. IEM. Medical Symposium, September 1960.

TABLE NO. 1

RECORD FILE STRUCTURE

KEY	FILE	RECORD FROM TO	BAND FROM TO	CHARACTERS PER MESSAGE	PER CELL	PER BAND	MESSAGES REQUIRED	ALLOCATED	NO. BANDS
	PROGRAM WORK AREA	000 024 025 049	000 099 100 199						100 100
	CODE-NAME-CODE- INDEX	050 061	200 245	75	825	11	5000	5000	46
1	CODE-NAME-NAME A	050 055	200 230	37	882	24	5000	5040	21
2	HEADING INFO A	053 057	231 230	70	840	12	1200	1200	10
3	CLINICAL CLASS A	057 059	231 237	49	882	18	1200	1260	7
4	SKIN TEST A	059 061	238 246	31	858	23	2400	2520	9
5	ROENTGENOLOGIC A	051 070	247 282	63	882	14	5000	5040	36
6	ECG A	070 075	283 302	28AV	868	31	12000	6250	20
7	BRONCHOGRAM A	073 077	303 308	38	874	23	1380	1380	6
8	URINALYSIS A	077 085	309 342	30	870	29	10000	9860	34
9	HEMATOLOGY A	083 094	343 377	37	888	24	10000	8400	35
A	BACTERIOLOGY A	044 106	378 427	44	830	20	10000	10000	50
B	FUNGAL SEROLOGY A	107 108	428 433	29	858	22	1200	1320	6
C	DRUG THERAPY A	108 127	434 511	43AV	860	20	15600	15600	78
D	MTB DRUG C	030 056	200 224	71	852	12	3000	3000	25
E	SUSCEPTIBILITY C	056 059	225 236	32AV	864	27	3000	3240	12
F	DRUG SERUM C	059 063	237 254	52	834	17	3000	3060	18
G	BACTERIAL C								
	SUSCEPTIBILITY								
	ALLERGY								
	INHALENTS-SCRATCH	061 062	246 251	39	858	22	1200	1320	6
H	ALLERGY								
	MIXES	063 064	252 258	50	850	17	1200	1190	7
I	RESP DIS QUEST	064 072	259 288	51	867	17	5000	5100	30

TABLE NO. 1 (Continued)

RECORD FILE STRUCTURE

KEY	FILE	RECORD FROM TO		BAND FROM TO		CHARACTERS PER MESSAGE PER CELL		PER CELL	PER BAND	MESSAGES REQUIRED ALLOCATED		NO. BANDS
J	SMOKING HISTORY	072	078	289	315	46	874	19	190	5000	5130	27
K	SPIROMETRY	079	122	316	490	63	882	14	140	30000	24500	175
L	SPIRO-INFT B	050	075	200	301	45	855	19	190	30000	19380	102
M	SPIRO-PREDICT B	075	093	302	374	32	864	27	270	30000	19710	73
N	SPIRO-TRACING											
	CHAR B	093	119	375	477	47	846	18	180	30000	18540	103
O	CLINICAL DIGEST	122	124	491	496	41	861	21	210	1200	1260	6
P	LUNG VOLUMES	124	126	497	506	68	884	13	130	1200	1300	10
Q	LUNG VOL-PRED B	119	121	478	484	47	846	18	180	1200	1260	7
R	TREADMILL	125	127	507	508	68	884	13	130	500	260	2
S	CO ₂ DIFFUSING											
	CAP B	121	122	485	489	XX	XXX	XX	XXX	500	XXX	5
T	BLOOD GASES	127	127	509	511	55	880	16	160	500	480	3
U	FLUROSCOPY B	122	122	490	491	27	864	32	320	500	640	2
V	SURGICAL TRETNT											
	B	123	124	492	499	51	867	17	170	1200	1360	8
W	DIAGNOSIS B	125	127	500	511	41AV	861	21	210	3000	2520	12
X	DIAGNOSIS CODE											
	TABLE C	060	067	255	268	10AV	880	XX	*8000	112000	112000	14

* An average of 8000 characters per band utilizing 14 bands for a total of 112,000 characters

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-1h The Relationship of Calcium In
Sweat To Calcium Intake and Calcium Balance

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio
and
LeRoy O. Matoush
Richard A. Nelson
Ernest Eugene Preston
L. Ross Hackler, Ph. D.

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-1h The Relationship of Calcium In Sweat To Calcium Intake
And Calcium Balance

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General
Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: C. Frank Consolazio, LeRoy O. Matoush, Richard A. Nelson, Ernest
Eugene Preston and L. Ross Hackler.

RCS MEDDE-288

Unclassified

In a review on dermal sweating Mitchell and Hamilton in 1949 reported calcium sweat values ranging from 8 to 265 mg/liter. This meant that under high sweating conditions, the sweat calcium could account for up to 30% of the daily total body loss. In all, three studies were performed to evaluate the sweat excretion in relation to the total calcium excretion and calcium balance. Study I was performed in a temperate environment, Study II at temperatures of 70, 85 and 100°F, and Study III, 16 days at 100°F.

The data presented in these studies show that an appreciable quantity of calcium is eliminated in the sweat, whether in a hot or a temperate environment. These findings are important since they show an additional calcium loss, which has not been reported in previous balance studies. Under conditions of these experiments,

where men exercised for 100 minutes a day, these values approximate 8.1 mg calcium/hour at 70°F, 11.6 mg/hr at 85°F and 20.2 mg/hr at 100°F. On low calcium intakes (441 and 580 mg/day) the sweat calcium ranged from 23 to 37% of the total daily output. It is suggested that in past balance studies on low calcium intakes, equilibrium may not have been attained, and men may have actually been in negative balance by 200 mg/day, since sweat losses were not considered. On the basis of this data it is suggested that calcium allowances for environments and conditions likely to cause sweating be increased to compensate for the losses due to sweating.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Metabolism and Nutrition

Sub-task No. 1-1h The Relationship of Calcium In Sweat to Calcium Intake and Calcium Balance

Description:

The purpose of this study was to see if dietary calcium requirements are increased due to calcium losses in sweat during profuse sweating conditions. Other problems to be investigated were whether or not the calcium concentration in the sweat is a reflection of the intake and whether or not the calcium excretion during exercise is higher in the heat or in a temperate environment.

Progress:

During the past year another study was completed to evaluate the relationship between calcium in sweat, calcium balance, and calcium requirements. In Study I performed in a temperate environment, the men exercised for three hours daily. In Study II, the men exercised for 100 minutes a day at temperatures of 70, 85 and 100°F. Study III, performed during the past year, was conducted for a total of 32 days, on 3 healthy young men. In the control period, the men were exposed to a temperature of 75°F for a period of 8 days. During the exposure to 100°F temperatures, the men were exposed for 16 consecutive days, and in the final recovery or adjustment period, the men again were exposed for 8 days at an environmental temperature of 75°F. The subjects exercised at a moderate rate for only 30 minutes daily, the remainder of the day being spent in sedentary type activities.

Summary and Conclusions:

This data suggests that sufficient calcium may be lost during heavy sweating conditions (up to 20 mg calcium/hour) and that this loss should be considered in establishing recommended allowances for calcium.

It has been observed that 7 men consuming 441 mg of calcium/day in a study extending for a total of 48 days, excreted 8.1, 11.6 and 20.2 mg/hr of calcium when living at 70, 85 and 100°F. This accounted for 21.8, 25.1 and 33.2% of the total calcium excreted.

These findings are important since they show an additional calcium loss, which has not been reported in previous calcium balance studies in the literature. It is questionable whether an individual, consuming a low calcium diet, ever really does attain calcium balance (equilibrium), under heavy sweating conditions.

It was observed that (a) the calcium excreted in sweat in men working at a light physical activity rate in extreme heat (100°F), is still fairly high after acclimatization, averaging 17 mg/hr after the first 4 days, and (b) that the daily total calcium in sweat will increase as the sweat rate increases. Therefore it would seem that the calcium requirements may be increased under these conditions.

Publications:

1. The Relationship of Calcium in Sweat to Calcium Intake and Calcium Balance. C. Frank Consolazio, LeRoy O. Matoush, Richard A. Nelson, Ernest Eugene Preston and L. Ross Hackler. Fed. Proc. 21, 2: A3082, 1962.

2. Calcium in Sweat and Its Possible Relation to Calcium Requirements.

C. Frank Consolazio, LeRoy O. Matoush, Richard A. Nelson, Ernest Eugene

Preston and L. Ross Hackler. USAMRNL Report No. 266, February 1962.

3. In press. Journal of Nutrition, September 1962 issue.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 1-11 Nutritional and Biochemical
Aspects of Carbohydrates

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio
and
Jack M. Iacono, Ph. D.
University of Cincinnati
Cincinnati, Ohio

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-11 Nutritional and Biochemical Aspects of Carbohydrates

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado, and the University of Cincinnati, Cincinnati, Ohio

1 July 1961 - 30 June 1962

Authors: C. Frank Consolazio and Jack M. Iacono

RCS MEDDH-288

Unclassified

As reported last year a manuscript has been prepared as one chapter in a new book to be entitled "Newer Methods of Nutritional Biochemistry with Applications and Interpretations" to be published by the Academic Press Inc., New York, Editor - Dr. Anthony A. Albanese. Final revisions have now been made and the book will be published in 1962.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-11 Nutritional and Biochemical Aspects of Carbohydrates

Description:

This report covers the preparation of a chapter on carbohydrates in a book entitled "Newer Methods of Nutritional Biochemistry with Applications and Interpretations" edited by Dr. A. A. Albanese and to be published by Academic Press, Inc. New York.

Progress:

A chapter on Carbohydrates was prepared and includes 11 sections as follows: (1) Introduction to Carbohydrates, (2) Classification of Carbohydrates (3) Carbohydrate Metabolism, (4) Carbohydrates in Disease, (5) Role of Carbohydrate in the Diet, (6) Normal Values of Carbohydrate Constituents, (7) Newer Concepts of Carbohydrate Metabolism, (8) Quantitative Chemical Analyses, (9) Paper Chromatography, (10) Tolerance Tests, and (11) Qualitative Tests.

During the past year the final revisions have been completed.

Summary and Conclusions:

A final draft was submitted for publication to the Editor, Dr. A. A. Albanese and then to the Academic Press, Inc. New York, with a 15 May 1962 press deadline.

List of Publications:

In press.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 1-2 The Energy Requirements of Men Exposed
To Solar Radiation and Heat (The Energy Expenditure
of Men Working In the Heat; The Effects of Training
And Acclimatization)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio
LeRoy O. Matoush
Richard A. Nelson
SSG Juan B. Torres

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-2 The Energy Requirements of Men Exposed to Solar Radiation And Heat

The Energy Expenditure of Men Working in the Heat;
The Effects of Training and Acclimatization

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: C. Frank Consolazio, LeRoy O. Matoush, Richard A. Nelson and Juan B. Torres

RCS MEDDH-288

Unclassified

It has been shown that there is an increase in energy requirements of men living and working in an extremely hot environment. The increased requirements are due to the added heat load imposed on the body by extreme environmental heat and by solar radiation.

Some of the main unanswered questions from these studies were whether the subjects were fully acclimated to the heat and whether the increase in energy requirements was due to insufficient training prior to the beginning of these experiments. In the present study the metabolic rate was measured under three strictly controlled levels of environmental temperature and humidity, replicated 4 times in a random order. The plan would permit the calculation of a factor for the increased metabolic rate due to each 15°F increment in environmental temperature.

Seven men performing standard exercise at 100°F had higher metabolic rates as compared to the same exercise at 85 and 70°F, with average increases of 11.4% for light activity, 13.3% for moderate activity, and 11.7% for heavier activity. The data indicates that these are not artifacts related to training or acclimatization.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-2 The Energy Requirements of Men Exposed to Solar Radiation And Heat

The Energy Expenditure of Men Working In the Heat;
The Effects of Training and Acclimatization

Description:

Recent work completed by this Laboratory has shown that there is an increase in the energy requirements of men living and working in extreme heat. This increase is due primarily to the heat load imposed on the body by solar radiation and the extreme environmental heat. The increased heat load is a result of the increased action of the blood in heat transport, increased action of the sweat glands, increased caloric loss due to sweat vaporization, and to increased body temperature.

The object of this study was to measure the energy cost at three levels of physical activity of men living both in an extremely hot (38°C), in a moderate (29.5°C), and in a cool environment (22°C). In this experiment the temperature was controlled by using a constant temperature and humidity (30%) room for all three phases. In past studies the daily temperature has not been accurately controlled, having a range of 15°C. Under these conditions one can rule out the effects of acclimatization to the heat and the effects of training.

Progress:

Metabolic rates were compared on 7 young men performing three levels of physical activity at three environmental temperatures of 70, 85 and 100°F.

This study indicates that as the environmental temperature increases there is also an increase in metabolic rate of men performing a fixed activity. It has been shown that there was a significantly higher metabolic rate for men working at 100 than 85 and 70°F. These increases averaged 11.4% for the light activity, 13.3% for the moderate activity and 11.7% for the heavier activity. Body temperatures also were significantly higher at 100 than at 85 and 70°F environments. The findings in this study indicate that the metabolic rate of a fixed physical activity is increased in the heat and that this increase is not due to acclimatization or training.

Publication:

1. Environmental Temperature and Energy Expenditure. C. Frank Consolazio, LeRoy O. Matoush, Richard A. Nelson and Juan B. Torres. USAMRML Report No. 267, 2 April 1962.
2. In press.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 1-4 The Nutritional Status of Military
(And/Or Civilian) Populations By Dietary, Biochemical
And Clinical Evaluation (Collaborative Studies With
Interdepartmental Committee On Nutrition for National
Defense, National Institutes of Health, U. S. Public
Health Service, Bureau of Indian Health)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division
Metabolic Division
Chemistry Division

1 July 1961 - 30 June 1962

Interdepartmental Committee On Nutrition
For National Defense (ICNND)
and

National Institutes of Health
and

US Army Medical Research and Nutrition Laboratory
and

U. S. Public Health Service
Bureau of Indian Health

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-4 The Nutritional Status of Military (And/Or Civilian)
Populations By Dietary, Biochemical and Clinical
Evaluation

Collaborative Studies with Interdepartmental Committee On Nutrition for National Defense, National Institutes of Health, U. S. Public Health Service, Bureau of Indian Health and U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Interdepartmental Committee On Nutrition for National Defense, National Institutes of Health, US Army Medical Research and Nutrition Laboratory, US Public Health Service, Bureau of Indian Health

RCS MEDDE-288

Unclassified

Through participation of USAMRNL personnel as team members and consultants, and through chemical analyses of food and blood and urine specimens, this Laboratory has assisted the Interdepartmental Committee On Nutrition for National Defense in surveys to assess the nutritional status of civilian and military populations in several areas of the world. Surveys conducted during the past year included Burma, Jordan, Uruguay and two American Indian groups living on reservations in Montana. Final reports have now been published for surveys conducted in previous years in Chile, Lebanon, Thailand, and Taiwan.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Metabolism and Nutrition

Sub-task No. 1-4 The Nutritional Status of Military (And/Or Civilian)
Populations By Dietary, Biochemical and Clinical
Evaluation

Collaborative Studies with Interdepartmental Committee On
Nutrition for National Defense, National Institutes of
Health, US Public Health Service, Bureau of Indian Health

Description:

The objectives of the surveys were (a) to characterize the nutritional level of the population, (b) to appraise the availability, source and use of foods, (c) to determine the dietary patterns, (d) to assess the technology and production of food, and its regulatory control, and (e) to review hospital and institutional feeding, in order to provide background for improvements in the nutritional health of the people of each group of individuals. Another objective of the surveys was to afford training and experience for personnel within the several responsible ministries and agencies concerned with nutrition.

Progress:

Final reports were prepared for the Lebanon, Chile, and Thailand studies. Reports are now in preparation, correlating the biochemical, dietary, clinical, dental, and food technology findings, for the other surveys.

A. American Indian Study:

In the American Indian study all of the biochemical procedures to evaluate nutritional status were done at this Laboratory. They included protein, albumin, hemoglobin, hematocrit, vitamin C, vitamin A, carotene; iron and RBC riboflavin -

done on 578 bloods. There were 434 urines collected and analyzed for thiamine, riboflavin, N¹MN, creatinine, urea nitrogen and total nitrogen. In addition chemical analyses of all the nutrients were also completed on the food composites and the individual welfare items. USAMRNL members of the survey team included C. F. Consolazio, Chief, Bioenergetics Division, who served as the Biochemistry Section Chief, and LeRoy O. Matoush, J. B. Torres, and R. A. Nelson.

B. Jordan:

Clinical, dietary, and biochemical examinations were made of samples of the population in Jordan in April, May and June 1962. These assessments included search for physical signs of malnutrition, and quantitative biochemical measurements reflecting nutriture with respect to various vitamins, iron, and other nutrients.

Approximately 9,500 - 10,000 examinees were included in one or another aspect of the survey and over 800 samples of blood and 700 specimens of urine were analyzed. Civilians, including recipients of UNRWA benefits, and military were included. Food intake was measured at 11 military installations in various areas of Jordan. C. F. Consolazio, Chief, Bioenergetics Division, USAMRNL served as Chief, Nutrition Section of the team.

C. Burma:

This survey took place from October - December 1961. Approximately 9,300 clinical examinations were completed with 50% being military personnel. In all 732 bloods and 684 urines were analyzed for the routine ICNND analyses. Additional analyses included 300 RBC transketolase, an index of thiamine intake, and 30% were found to be in the deficient range. The status of vitamin E, using the RBC hemolysis test was completed on 197 samples, showing that 11%

were deficient and 130 samples of urine were analyzed for their vitamin B₆ content; 60.7% of the samples were found to have low vitamin B₆ excretions. Tryptophan load tests, performed on 55 men, showed that 43% of the subjects had marked xanthurenic acid excretions, which is an indication of deficient vitamin B₆ intake.

USAMRNL members of the survey team were Howerde E. Sauberlich, Chief, Chemistry Division, who served as the Biochemistry Section Chief, and Captain George E. Bunce, MSC, Chemistry Division.

D. Uruguay:

A survey was conducted in Uruguay during the months of March and April 1962 by a joint Uruguayan-American team sponsored by the ICNND. 5944 persons were examined, including 573 military and 5371 civilians. Goiter was the most striking clinical physical finding noted with the incidence ranging from 27.7% to 69% in various locations. Biochemical determinations were performed with the greatest "deficiencies", as defined by ICNND criteria, noted in the serum vitamin C levels. Complete analyses of clinical and biochemical data, including food composites, are in the process. Food technology has not been developed to the optimum. The agricultural land is low in phosphates and needs fertilization.

Special studies conducted during this survey included evaluation of selected older individuals for diabetes and arteriosclerosis. The determinations of contraction time of the Achilles reflex (Lawson test) for evaluation of thyroid function and its relationship to the presence of goiter was carried out on 1156 individuals. Data evaluation of these special studies is not complete.

Lt. Colonel John E. Canham, MC, Chief, Metabolic Division, USAMRNL, and Colonel John S. Zelenik, MC of Fitzsimons General Hospital were members of the clinical survey team, and Lt. Colonel Marion E. McDowell, MC, Commanding Officer, USAMRNL participated in the prior planning in Montevideo for the survey.

E. Lebanon:

A nutrition survey was performed in the Republic of Lebanon during March and April of 1961 and the final report was completed and published during this year. The survey team was composed of Lebanese, American, French, and United Nations Agency personnel who evaluated the availability of food, food technology, dietary pattern, and the physical and biochemical status of samples of the military, civilian, and refugee segments of the population of the country. The USAMRNL member of the clinical team was Major William T. Nunes, MC, Metabolic Division, USAMRNL. Over 8600 individuals were examined, including the military (2284), refugee (1485) and civilian (4851) groups. It was found that the caloric intake of the average Lebanese adult is adequate, but a deficit in caloric allowance was found in the refugee. Thyroidal enlargement was the greatest sign of nutritional inadequacy which was confirmed by the finding of low urinary excretion of iodine. Dietary, biochemical and clinical evidences of widespread deficiency of riboflavin were encountered in all segments of the population and throughout the entire country. Clinical signs of hypovitaminosis A were found scattered throughout all groups studied and in the armed forces there was additional evidence of inadequate dietary intake of this vitamin. Recommended corrective measures include: iodation of salt, increased production, use and availability of riboflavin containing foodstuffs,

and agricultural planning with attention to improving the seasonal deficiency of vitamin A in the dietary.

F. Completed Reports:

Final reports of other ICNND surveys were completed during this reporting year. They consist of the report on a nutrition survey of Chile conducted in March - June 1960 in which Lt. Colonel Marion E. McDowell, MC, of USAMRNL participated as a clinician, and reports of the surveys of Thailand and of Taiwan in which USAMRNL members of the Nutrition Section of the team were C. Frank Consolazio and SSG Juan B. Torres.

G. Resume of Foreign Officer Training In Nutrition and Nutritional Research Techniques:

During the past year three foreign officers, one from Taiwan, one from Pakistan, and the third from Iran, received training at USAMRNL in the field of nutrition and in the various clinical, biochemical and dietary techniques required to evaluate the nutritional status of a population.

Conclusions:

Continued participation in nutrition surveys sponsored by the ICNND and other cooperating agencies is most desirable because of (1) the valuable experience gained in techniques of assessment of nutritional status in population groups, (2) the opportunities to observe and study particular nutritional problems less common in this country, (3) the valuable service rendered both to the civilians and to military groups in other countries, which incidentally serves as a concrete example of U. S. goodwill, and (4) because of the knowledge gained pertinent to global medicine.

Specific conclusions are deferred to publication of the comprehensive survey reports by ICNND, or of special reports from this Laboratory.

Publications:

1. Republic of China. Nutrition Survey of the Armed Forces Sept - Oct 1960. A preliminary report of the ICNND, July 1961.
2. The Kingdom of Thailand, Nutrition Survey, October - December 1960. Report of the ICNND, February 1962.
3. Republic of Lebanon. Nutrition Survey February - April 1961. A report of the ICNND, May 1962.
4. Chile Nutrition Survey March - June 1960. A report of the ICNND, August 1961.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 1-4a The Nutritional Status of Military
Populations By Dietary, Biochemical and Clinical
Evaluation (Nutritional Evaluation of A Normal
Military Population)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio
and
Ralph Shapiro
Gerhard J. Isaac
Laurence M. Hursh, Colonel, MC

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-4a The Nutritional Status of Military Populations By Dietary, Biochemical and Clinical Evaluation

Nutritional Evaluation of A Normal Military Population

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: C. Frank Consolazio, Ralph Shapiro, Gerhard J. Isaac and Laurence M. Hursh.

RCS MEDDH-288

Unclassified

Anthropometric and biochemical data, previously gathered on military personnel, including Army, Navy and Air Force units in the Denver, Colorado Area, and described in last year's Annual Progress Report (1 July 1960 to 30 June 1961) has been published in detail in USAMRNL Report No. 260, 14 August 1961, and is now in press, in re-edited form, in the American Journal of Clinical Nutrition. No new data have been collected in the past year. Published data include the ages, heights, body weights, blood pressures, and skinfold thickness measurements of 4144 enlisted men, chemical analyses (on every 5th man examined) consisting of hemoglobin, hematocrit, plasma protein, vitamin C, vitamin A, carotene, fat partitions in the blood, and urine analyses for thiamine, riboflavin and N¹-methyl-nicotinamide and calculated/gm of creatinine excreted. Data were classified into 8 separate age groups and then compared to ICNND and to other normal data in the literature.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-4a The Nutritional Status of Military Populations By Dietary, Biochemical and Clinical Evaluation

Nutritional Evaluation of A Normal Military Population

Description:

Recent studies by Pollack in Formosa in 1956 and by ICNND survey teams in 23 countries of Asia, Africa, Europe and South America have stressed the importance of obtaining anthropometric and biochemical data on military personnel for purposes of comparison among surveys and with other groups.

Another problem which is of concern to the military is that of obesity, particularly among individuals in the older age groups, who are engaged in light or sedentary activities.

The object of this study was to gather anthropometric and biochemical information on selected military personnel of all ages living in the Denver, Colorado area. This would help establish normal standards for the evaluation of the nutritional status of military populations.

Progress:

During the current year the data has been further analyzed and reviewed, the manuscripts edited and a detailed report published as USAMRNL Report No. 260, 14 August 1961. In addition, the manuscript has been further edited and is in press in the American Journal of Clinical Nutrition. No new data has been collected in the past year, though plans are being made for resumption of annual nutrition surveys of selected Army Camps and Stations.

Summary and Conclusions:

The nutritional status of military personnel from the army, navy and air force in all age groups were evaluated both anthropometrically and biochemically. Anthropometric measurements were collected on 4144 enlisted men and included ages, heights, body weights, blood pressures and skinfold thicknesses. Biochemical analyses in bloods and urines were done on approximately 800 samples. The data was analyzed by age groups and compared to ICNND and other normal data in the literature.

The following conclusions were noted in the data:

1. There was a decrease in height with age (175.5 - 172.0 cm), the tallest men being in the youngest age group.
2. The body weights increased with age up to age 45 years (69.9 - 77.3 kg).
3. There was an increase in 3 of the 4 skinfold thickness measurements with an increase in age (chest, abdomen and sub-scapular).
4. Of all the blood and urine data only total cholesterol, total lipid and phospholipid values showed an increase with age.
5. 11.3% of the thiamine excretion/gm of creatinine were in the deficient ICNND range, (It should be noted that 95% of these values were on married men living and eating at home.) and 16.0% of the plasma protein values were in the deficient ICNND range of below 6.0 gm.

List of Publications:

1. Nutritional Evaluation of a Normal Military Population. C. F. Consolazio, R. Shapiro, G. J. Isaac, and L. M. Hursh. USAMRNL Report No. 260. 14 August 1961, Denver 30, Colorado.
2. In Press. Am. J. of Clinical Nutrition.

FINAL REPORT

Project No. 6X60-G1-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 1-10 The Acceptability of A High
Protein-High Calorie Chocolate Drink

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio
SSG Juan B. Torres
Marion E. McDowell, Lt. Col. MC

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-10 The Acceptability of A High Protein-High Calorie Chocolate Drink

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: C. Frank Consolazio, Juan B. Torres and Marion E. McDowell.

RCS MEDDH-288

Unclassified

The U. S. Army Medical Service in cooperation with the U. S. Army Quartermaster has developed a high calorie-high protein beverage powder for use as a food supplement primarily at battle group aid stations. This food supplement, that can be served either warm or cold, was field tested for acceptability using a 5 category scale, at the "Exercise Lava Plains" Yakima, Washington from May 8 - 22, 1961. Four ounces of the dried powder, made up in 8 ounces of water, was consumed by 447 simulated and 24 actual casualties. Preliminary evaluation of the data show the food supplement to be highly acceptable. In the group of 471 individuals only 13 men disliked the product. Very few, if any, adverse after effects were noted. USAMRNL Report No. 263, 18 September 1961 was published recommending adoption of the supplement for use at forward medical installations.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-10 The Acceptability of A High Protein-High Calorie Chocolate Drink

Description:

The main object of this study was to evaluate the acceptability of a high Calorie-high protein food supplement, under simulated combat conditions. This instantized dry powder contains 87.6% whole milk, 8.3% sugar, 2.3% cocoa, 1.5% vanilla sugar, and 0.23% instant coffee. Four ounces of the dry powder contains approximately 590 Calories. The supplement was available in 4 ounce plastic containers and in one pound cans.

Background information relative to the development of this ration supplement is summarized in USAMRNL Report No. 263, 18 September 1961.

Progress:

The general plan was to test the acceptability of this food supplement on simulated and actual casualties during the field test (Exercise Lava Plains). Simulated casualties were studied at 5 battalion aid stations, at three clearing stations (MASH hospital) and at the 8th Field Hospital. The actual casualties were studied at the 60th Field base hospital.

Troops were questioned daily on whether they liked or disliked the chocolate supplement, using a 5 category acceptability rating scale. In addition they were questioned on the subjective and objective responses that

may be due to the chocolate supplement (nausea, vomiting, diarrhea, constipation, etc.). Information also included (a) the maximum quantity to be issued at one time, without ill effects, (b) the best temperature for consumption, (c) whether the instantized dry powder was easily prepared and (d) whether the plastic containers or the one pound tins were practical under field conditions.

This field study was completed in May 1961 and the report was prepared and published during this reporting period, in September 1961.

Summary and Conclusions:

The acceptability of the high Calorie-high protein food supplement was evaluated on a total of 471 men (447 simulated and 24 actual casualties) engaged in "Exercise Lava Plains" at Yakima, Washington from May 8 - 22, 1961. Of the 447 simulated casualties questioned only 11 men or 2.5% expressed dislike for the product. The only undesirable side effect, presumably attributable to the food supplement, was the stomach upset reported by two of the men. The acceptability is increased when the food supplement is served warm in cold weather and cold in warm weather.

The high Calorie-high protein food supplement is highly recommended for use at battalion aid stations, clearing stations and field hospitals for casualties.

Publications:

Field Test of A High-Calorie, High-Protein Beverage Powder for Use As A Ration Supplement at Forward Medical Stations. C. Frank Consolazio, Juan B. Torres and Marion E. McDowell. USAMRNL Report No. 263, 18 September 1961.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 1-11 Nutrient Losses in Sweat

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio
LeRoy O. Matoush
Richard A. Nelson
John E. Canham, Lt. Col. MC
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-11 Nutrient Losses in Sweat

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: C. Frank Consolazio, LeRoy O. Matoush, Richard A. Nelson, John E. Canham, and Richard S. Harding.

RCS MEDDH-288

Unclassified

In a 16-day study on men exposed to 100°F in 70% relative humidity, complete mineral balances (sodium, potassium, magnesium, phosphorus and iron) and nitrogen balances were conducted on men while on a constant daily intake (except salt) and constant daily activity. In addition to 4-day fecal and urine collections, sweat samples were collected daily both morning and afternoon and were analyzed for sodium, potassium, magnesium, iron, nitrogen and phosphorus. The $7\frac{1}{2}$ hour excretion in sweat of the various minerals measured, ranged from 2.37 - 10.8 gm for sodium, 9 - 28 mg for magnesium, 0.65 - 1.77 mg for iron, 3.4 - 9.9 mg of phosphorus, and 516 - 863 mg for potassium. The sweat losses accounted for 5.3% of the total magnesium losses, 25% of potassium losses and approximately 10% of iron losses. Nitrogen excretion in sweat ranged from 1.55 - 2.38 gm/ $7\frac{1}{2}$ hour period accounting for 12 - 18% of the total daily intake. Phosphorus losses in sweat were negligible.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-11 Nutrient Losses in Sweat

Description:

The primary purpose of this experiment is to measure the quantity of various nutrients and metabolites excreted in the sweat of men exposed to increased environmental temperatures, and to evaluate the relation of this sweat loss of nutrients to acclimatization, level of intake, state of balance, etc. Such studies are of importance since losses by sweat may be considerable, but have frequently been ignored in past studies published in the literature.

Progress:

In a 16-day study, 3 men were exposed to 100°F and 70% relative humidity daily for 7½ hour periods, and sweat collections were made. Physical activity was kept at a minimum, the men working only 30 minutes a day. Food composites, urine and fecal collections were made on each man for four 4-day periods. Daily morning and afternoon sweat rates and sweat samples were collected and analyzed so that balances could be computed including and excluding the sweat losses. Analyses of Na, K, Mg, Fe, total nitrogen and phosphorus have been completed on the food composites, urine, feces and sweat samples, and freezing-point determinations have been done on the sweat. Creatinine, urea, uric acid and non-protein nitrogen are now being analyzed in the sweat samples.

Summary and Conclusions:

In a 16-day study of 3 men exposed for $7\frac{1}{2}$ hours daily to 100°F and 70% relative humidity the $7\frac{1}{2}$ hour excretion in sweat of the various minerals measured ranged from 2.37 - 10.8 gm for Na, 9 - 28 mg for magnesium, 0.65 - 1.77 mg for iron, 3.4 - 9.9 mg for phosphorus, and 516 - 863 mg for potassium (for the $7\frac{1}{2}$ hour period). The sweat losses accounted for 5.3% of the magnesium losses, 25% of the potassium, approximately 10% of the iron, and only 0.8% of the phosphorus.

The range of nitrogen excretion in sweat in this study was from 1.55 - 2.38 gm in a $7\frac{1}{2}$ hour period. This accounted for 12 - 18% of the total daily intake. Protein nitrogen excretion in sweat ranged from 144 - 250 mg for a $7\frac{1}{2}$ hour period, creatinine from 18 - 31 mg and urea nitrogen from 0.52 - 1.03 gm for a $7\frac{1}{2}$ hour period.

These data suggest that a considerable quantity of minerals and nitrogen may be excreted in sweat, and that these losses should be considered when computing balances and estimating requirements.

Publications:

None. Two manuscripts are in preparation for publication.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 1-12 Nutrition Study of Men In
A Fallout Shelter

A Collaborative Study With the Naval Medical
Research Institute, Bethesda, Maryland
And USAMRNL

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

Robert Van Reen, Ph. D.
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-12 Nutrition Study of Men In A Fallout Shelter

A Collaborative Study with the Naval Medical Research
Institute, Bethesda, Maryland and USAMRNL

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General
Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Robert Van Been, C. Frank Consolazio, David Minard, LeRoy O. Matoush
and Richard A. Nelson

RCS MEDDH-288

Unclassified

In February of 1962 the U. S. Navy Medical Research Institute conducted a study on 100 navy recruits, living in a fallout-shelter for two weeks. During the study the men were at first allowed approximately 1480 and then 2000 kCalories/day, which consisted primarily of a commercial cracker under consideration as an austere ration for shelter stock-piling.

Fasting bloods and 6-hour urines were collected on 25 of the men before and after they came out of the shelter. In addition 24-hour urines were collected.

The chemical analyses conducted at the USAMRNL in a collaborative effort included vitamin A, carotene, total protein, albumin, hemoglobin, hematocrit, vitamin C, RBC riboflavin and RBC transketolase on the blood; and thiamine, riboflavin, niacin, pyridoxine and

creatinine in the fasting and 24-hour urines. The data is now being processed for a future publication. Overall acceptability of the crackers (96%) was better than had been expected from observations on the 2-day pre-test study. Higher values for total plasma protein, hematocrit, and hemoglobin after the test suggested the occurrence of a degree of dehydration. Weight loss occurred with an average of 5.0 lbs lost during the 8 days at 1480 Calories, but only 0.4 lbs mean weight loss during the last 6 days at 2000 Calories/man/day.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 1-12 Nutrition Study of Men In A Fallout Shelter

A Collaborative Study With the Naval Medical Research
Institute, Bethesda, Maryland and USAMRNL

Description:

A study of the nutritional status of a randomly selected sample from approximately 100 naval recruits maintained for two weeks in a fallout shelter on a rather austere ration being considered for stock-piling for such shelters. This study, carried out jointly by this Laboratory and the National Naval Medical Center, took advantage of the opportunity to make these observations during a habitability study (the main purpose of the test) in a protective shelter at the National Naval Medical Center, Bethesda, Maryland, between 17 February and 3 March 1962.

Progress and Summary:

The main constituent of the diet was a commercial cracker (National Biscuit Co.) which contains wheat flour, cane sugar, corn flour, corn sugar, cotton seed oil, soya flour, salt, leavening, vitamin B₁, and a preservative. Two meals were planned. The first or cold meal consisted of 172 gm of crackers, 20 gm of jam, and sugar and Pream for coffee. This meal would provide about 890 Calories. The second or hot meal was to provide 172 gm of crackers, about 90 gm of a condensed soup, 16 gm of peanut butter, and sugar and Pream. This meal would provide 960 Calories, for a total daily intake of 1850 Calories.

In a two day pre-test, consumption of the crackers was much less than expected, particularly at the hot meal with soup. For this reason, the issue of crackers at the start of the 14-day test was reduced to 86 gm for the second meal, thus providing about 1480 Calories/man/day. A mean weight of 5.0 lbs occurred during the first 8 days of the test with this reduced caloric intake. The issue of crackers was then increased to the planned level for the remainder of the test. Weights were fairly stable on the higher caloric intake, with an average weight loss of only 0.4 lbs during the last 6 days of the test.

Acceptability of the crackers was better than had been expected from the observations on the 2-day pre-test study. An overall acceptability of 96% was found, with no pronounced difference in acceptability when the issue of crackers was increased for the latter part of the study.

A study of the nutritional adequacy of the ration was made. Blood and urine samples were obtained from 25 subjects two days before entering the shelter, and second samples taken from the subjects within two hours of emerging from the shelter and before any normal foods were consumed. The following assays were made at the U. S. Army Medical Research and Nutrition Laboratory at Denver: plasma vitamin A, carotene, and vitamin C, hemoglobin, hematocrit, RBC riboflavin, blood transketolase activity, urinary creatinine, N¹methylnicotinamide, thiamine, riboflavin, and vitamin B₆.

Preliminary Conclusions:

A comparison of pre-test and post-test values indicates that there were higher values for total plasma protein, hematocrit, and hemoglobin after the test. This might be partially due to a degree of dehydration. There were no overt signs of vitamin deficiency; however, blood levels of carotene, riboflavin,

vitamin B₆, and vitamin C were all lower at the end of the test than initially, though they were still well above lower limits of normal. The data is being further evaluated, and another test is planned for July 1962 in which water balance will be included.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-7 Tissue and Blood Enzymes of Rats Eating
Irradiated Foods

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

Nicholas Raica, Jr., Ph.D.
and
H. E. Sauberlich, Ph.D.

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-7 Tissue and Blood Enzymes of Rats Eating
Irradiated Foods

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Nicholas Raica, Jr., and H. E. Sauberlich

RCS MEDDH-288

Unclassified

No new studies have been initiated on this project
during the past year due to transfer of personnel.

Because of its importance to nutrition and irradiated
foods, renewed efforts will be made to study the changes
brought about in enzymatic activity by irradiated foods.

Successful field applications were made of the
erythrocyte transketolase assay (thiamine) and eryth-
rocyte cell fragility (vitamin E) methods in the
nutritional survey in Burma this past year.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-7 Tissue and Blood Enzymes of Rats Eating Irradiated Foods

Description and Summary:

No new studies have been initiated on this project during the past year due to transfer of personnel. Because of its possible importance to nutrition and irradiated foods, renewed efforts will be made to study the changes brought about in enzymatic activity by irradiated foods. Emphasis will be placed on total liver lipids as well as mitochondrial lipid changes due to irradiated foods. The cytochrome oxidase system will be the enzyme system of choice since it is the most affected by irradiated diets.

Successful field applications were made of the erythrocyte transketolase assay (thiamine) and erythrocyte cell fragility (vitamin E) methods by members of the Chemistry Division during their participation in the nutrition survey in Burma, sponsored by the Interdepartmental Committee on Nutrition for National Defense, this past year.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-8 Supervision of OTSG Irradiated Food Contracts

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

Nicholas Raics, Jr., Ph.D.
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-3 Supervision of OTSG Irradiated Food Contracts

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Nicholas Raica, Jr., M. E. McDowell and H. E. Sauberlich

RCS MEDDH-288

Unclassified

Technical assistance and liaison between TSGO and
SGO contractors has continued. Current progress reports
and final reports have been received, duplicated and
distributed to SGO and QMF&CI.

Scientific meetings were attended with participation.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. C2 Metabolism and Nutrition

Sub-task No. 2-8 Supervision of OTSG Irradiated Food Contracts

Description and Summary:

Supervision of contracts let by OTSG regarding irradiated foods was provided. Monitoring, printing and distribution of progress or final reports were also provided.

Papers on irradiated food studies were presented at 1) Food and Agriculture Organization of the United Nations Technical Meeting on the Evaluation of the Wholesomeness of Irradiated Foods, Brussels, Belgium, in October 1961, 2) MEND Symposium on "Nutrition in National Defense," Denver, Colorado in May 1962.

List of Publications:

1. "Studies of the Wholesomeness and Nutritional Adequacy of Irradiated Foods." Summary of Results of Participation in the QM Food Irradiation Sterilization Program in Proceedings, Seventh Contractors' Meeting, QMC Radiation Preservation of Foods Project, June 1961.

2. McDowell, M. E. Review of the U. S. Army's Irradiated Food Wholesomeness Program (presented at the FAO-WHO-AEC Technical Meeting on the Evaluation of Wholesomeness of Irradiated Foods, Brussels, Belgium, 23-30 October 1961). U.S. Army Med. Rsch. & Nutr. Lab. Rpt. #268, 1962.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-13 Quantitative Determination of Antituberculous Drugs
in Body Fluids (Study No. 2 The Chemistry and
Metabolism of PAS-C)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

Z. Z. Ziporin, Ph.D.
W. C. Morse, Lt. Col., MSC
and
J. S. Chambers, M.S.

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-13 Quantitative Determination of Antituberculous Drugs
in Body Fluids

Study No. 2 The Chemistry and Metabolism of PAS-C

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Z. Z. Ziporin, W. C. Morse and J. S. Chambers

RCS MEDDH-288

Unclassified

The administration of PAS-C (para-aminosalicylic acid-ascorbate) to tuberculous patients, in place of PAS acid or sodium PAS has resulted in distinct advantages. Since PAS-C has not been described in the literature as a well-defined chemical entity, it was necessary to characterize the compound.

By the use of chemical and physical methods of analysis, PAS-C appeared similar to PAS. The laboratory synthesis of PAS-C using radioactive ascorbic acid resulted in a product which contained such minute amounts of ascorbic acid as to rule out the possibility of a definite compound as PAS-C, and explained the inability of physical and chemical means to distinguish the free acid from the PAS-C.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-13 Quantitative Determination of Antituberculous Drugs in Body Fluids

Study No. 2 The Chemistry and Metabolism of PAS-C

Description:

Pilot studies have shown that PAS-C is better tolerated by tuberculous patients as compared with PAS. Furthermore, optimum blood levels of PAS can be achieved with lower doses of PAS-C than with PAS. It is of interest to understand the mechanisms whereby PAS-C and PAS are metabolized by the body.

Progress:

By use of spectrophotometric absorption curves, Bratton-Marshall chemical assay, carbon-hydrogen analysis, paper chromatography, derivative formation and titration with sodium nitrite, PAS was the same as PAS-C. However, the melting points were different, though these tended to change with time, and eventually gave the melting point for the PAS acid.

Though a figure of 3-4% ascorbic acid has been reported for PAS-C, this could not be confirmed by subsequent assay. Therefore, a PAS-C was synthesized in this laboratory using ascorbic acid C¹⁴. The results show that small amounts of ascorbic acid are present in the first crop of crystals. This product has the melting point of PAS-C. With repeated recrystallization, the specific activity

Sub-task No. 2-13, Study No. 2

decreases and the melting point reaches that of free PAS acid. It was calculated that there was 1 molecule of ascorbic acid to 64,000 molecules of PAS for each disintegration per minute. This gave a maximum of 20 molecules of ascorbic acid to 64,000 molecules of PAS.

Summary and Conclusions:

Ascorbic acid does not react with PAS to form a definite chemical entity. Its different biological activity may be related to the purity of the PAS, as it is recrystallized, or to the change in crystalline structure of the PAS as it is recrystallized from a solution containing ascorbic acid.

List of Publications:

None at this time.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-18 Vitamin K and Irradiated Foods
(Study Nos. 1,2,3 Vitamin K-Vitamin A
Interrelationship in Prothrombin Levels
and Carotene Utilization in the Chick
and Rat)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

Nicholas Raica, Jr., Ph.D.
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-18 Vitamin K and Irradiated Foods

Study Nos. 1,2,3 Vitamin K-Vitamin A Interrelationship in Prothrombin Levels and Carotene Utilization in the Chick and Rat

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Nicholas Raica, Jr., T. I. Norris, C. L. Combs and H. E. Sauberlich

RCS MEDDH-288

Unclassified

Studies have been continued on the susceptibility of various vitamin K preparations to gamma irradiation. Data indicate that, when sealed under vacuum, vitamin K₁ and vitamin K₅ are stable to 100 megarads, while activity of vitamin K₃ is reduced by 40%.

High levels of vitamin A (10,000 - 50,000 IU/day) decrease prothrombin levels in chicks on vitamin K-free diet. Normal prothrombin levels are nearly restored by small daily doses of vitamin K. It does not appear that vitamin K requirements are increased by high levels of vitamin A.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-18 Vitamin K and Irradiated Foods

Study Nos. 1,2,3 Vitamin K-Vitamin A Interrelationship in Prothrombin Levels and Carotene Utilization in the Chick and Rat

Description and Summary:

Continuation of the studies on the effect of ionizing radiations on vitamin K is being pursued with pure vitamin preparations and the chick assay method. The vitamins under study are K₁, K₃, K₅, and KsII.

Preliminary data show that vitamin K₁, sealed under vacuum and irradiated to 94 megarads, retains its activity. Similarly prepared and irradiated vitamin K₅ also retains its activity when irradiated to 100 megarads. Vitamin K₃, on the other hand, loses about 20% of its activity when irradiated to 50 megarads, and 40% at 100 megarads.

On a weight basis, vitamin K₃ is about 2.5 times more active than vitamin K₁ or vitamin K₅ in the chick.

Similarly prepared samples sealed under nitrogen or oxygen will be assayed in the near future.

To supplement the chick assay method, the spectrophotometric and thin layer chromatographic methods are being investigated for evaluating changes produced by irradiated. Sufficient data have not been accumulated for comment at this time.

Sub-task No. 2-18, Study Nos. 1,2,3

Preliminary data from studies of vitamins A and K in the chick indicate that while high levels of vitamin A do decrease prothrombin levels in the absence of vitamin K, there does not seem to be an increased vitamin K requirement. 10,000 IU vitamin A per day is as effective as 20,000 or 50,000 IU per day. Liver vitamin A storage indicates that 20,000 IU per day in a single oral dose is the maximum amount absorbed.

Stabilized vitamin A added to the diet is well absorbed even at 5 million units per kilogram of diet. Hypervitaminosis A is evident by gross and microscopic examination within two weeks. To rule out the possibility that the antioxidants BHA and BHT present at such high levels of vitamin A were contributing to the toxicity, they were tested individually. There was decreased growth within one week at 0.5% BHA or BHT and at 0.1% BHT. However, there were no outward symptoms or decreased prothrombin levels as with high vitamin A dosages. Additional interest in these tests may be in the possible toxicity of BHT. The test dose is 0.1% or 10 times the maximum allowable concentration.

Studies with rats have been initiated to determine whether or not the vitamin K-vitamin A relationship is still observed when carotene serves as the source of vitamin A. No reportable data have been collected at this reporting date.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-19 Nutritional Potentialities of Algae
(Study Nos. 1-6 Studies of the Protein
Quality and Digestibility of Algae)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

G. A. Leveille, Ph.D.
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W. C. Goad, Sp4
and
H. J. Schneider, Pfc.

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-19 Nutritional Potentialities of Algae

Study Nos. 1-6 Studies of the Protein Quality
and Digestibility of Algae

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: G. A. Leveille, H. E. Sauberlich, J. A. Mielbrock,
W. C. Goad and H. J. Schneider

RCS MEDDH-268

Unclassified

Analytical and animal studies have been employed to appraise the nutritive value of algae. The results of the analytical studies demonstrate a high nutritive potential for algae. The amino nitrogen of algae has been found to represent only 80% of the total nitrogen. Gas chromatographic analyses of algae lipid have shown several unidentified peaks.

Digestibility studies in rats have indicated that dietary enzyme supplements may be of value in improving the poor digestibility of algae.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-19 Nutritional Potentialities of Algae

Study Nos. 1-6 Studies of the Protein Quality
and Digestibility of Algae

Description:

The development of algae for use as a food would enable the rapid growth of large quantities of a nutritious material. In view of the possible use of algae in emergency feeding, space feeding and its possible supplementary value, studies have been designed to develop a palatable and nutritionally adequate product from algae.

Progress:

Progress since the last report on this project has included digestibility studies of algae protein and the influence of various amylolytic enzymes on protein digestibility. Considerable effort has also been expended on analytical studies of various algae. Various species of algae have been analyzed for protein, fat, ash, amino acids and various vitamins.

Digestibility studies in young rats demonstrated the protein of algae to be rather poorly digested (apparent digestibility - 59%). The addition of certain amylolytic enzymes appeared to improve digestibility. The enzymes which appeared most effective were diastase and alpha amylase (apparent digestibility 73.8 and 70.6%, respectively). Further studies along these lines are anticipated.

Sub-task No. 2-19, Study Nos. 1-6

The data obtained indicate that algae has a very high nutritional potential; it is quite high in crude protein (30-60%), contains substantial amounts of the various vitamins and a considerable quantity of ash. However, data obtained from differential nitrogen analyses demonstrate that the protein content of algae is not as high as the total nitrogen analyses indicate. Twenty percent of the total nitrogen of algae has been shown to be non-amino acid nitrogen (amide, ammonia and humin nitrogen). The fat content of algae which contributes to its high caloric content is also not all available; considerable proportions of the algae lipid have been shown to be chromogenic material which would be of little value nutritionally. Gas chromatographic analyses of algae lipids have revealed a number of unidentified peaks which could be unsaturated fatty acids containing odd numbers of carbon atoms or possibly branched chain fatty acids. Studies are presently under way to identify these peaks.

Amino acid analyses of the algae have indicated adequate contents of most of the amino acids. However, all of the algae studied to date have been found to be low in sulfur-containing amino acids. This observation supports the results of the animal studies previously reported which demonstrated significant growth stimulations as a result of methionine supplementation of algae diets for rats and chicks.

Algae have been grown in this laboratory with C^{14} in the culture media resulting in cells containing uniformly labeled algae. It is

Sub-task No. 2-19, Study Nos. 1-6

planned that this material will be employed for further and more intense studies of algae utilization by experimental animals.

At present, studies are being directed at extending the data thus far obtained and overcoming some of the problems observed. The factors being considered are listed below:

1. Continued analytical studies of different species of algae and of algae grown under varying conditions.
2. Identification of the components of algae lipids by gas chromatography.
3. Improvement of the nutritional value and palatability of algae by
 - a. Processing
 - b. Supplementation
 - c. Improving digestibility
 - (1) Use of new species of algae
 - (2) By pre-treatment utilizing mechanical (ultra sonic waves), chemical (alkali or acid), and biological (enzymatic) methods
 - d. Use of isotopically labeled algae for studies of digestion, absorption and metabolism of the nutrients of algae

Summary and Conclusions:

The analytical data thus far obtained have demonstrated a high nutritive potential for algae. The addition of enzymes to algae diets in order to improve digestibility has shown some promise since certain enzymes did appear to improve digestibility.

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Sub-task No. 2-19, Study Nos. 1-6

The data thus far obtained are inadequate to form conclusions; it does appear that algae, with proper treatment, might be employed as a food source.

List of Publications:

1. Leveille, G. A., H. E. Sauberlich and J. W. Shockley. The protein value and amino acid deficiencies of various algae for growth of rats and chicks. J. Nutrition 76: 426, 1962.
2. Leveille, G. A., H. E. Sauberlich and J. A. Edelbrock. The influence of enzyme supplementation on the digestibility of algae. U. S. Army Med. Rsch. & Nutr. Lab. Rpt. #259, 20 June 1961.
3. Leveille, G. A., H. E. Sauberlich and M. E. McDowell. The nutrient content of various algae and the amino acid adequacy for growth of rats and chicks. To be published in Report of the Symposium-Workshop on Biologistics for Space Systems, Dayton, Ohio, 1-3 May 1962.

FINAL REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-20 Studies on the Metabolism of Pentoses
(Study No. 2 Metabolism of D-glucuronolactone-6-C¹⁴
and D-glucuronic-6-C¹⁴ Acid in Man)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-20 Studies on the Metabolism of Pentoses

Study No. 2 Metabolism of D-glucuronolactone-6- C^{14}
and D-glucuronic-6- C^{14} Acid in Man

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
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1 July 1961 - 30 June 1962

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Studies of body composition and the use of C^{14} isotopes have resulted in a method for stating the actual utilization of ascorbic acid by healthy men. After ingesting a single 20 μ c quantity of D-glucuronolactone-6- C^{14} , the urine of healthy men contains C^{14} activity which can be isolated and found in the highly purified phenylhydrazine derivative of ascorbic acid. Such activity is not found following the ingestion of D-glucuronic-6- C^{14} acid, indicating that the above lactone, but not its acid, can function as a source of ascorbic acid. In subjects who ingest 20 μ c of L-ascorbic-1- C^{14} acid, the daily urinary oxalate arising from metabolism of the labeled ascorbate is subsequently excreted as a constant proportion of the total C^{14} activity remaining in

Sub-task No. 2-20, Study No. 2

the body. Thus, it can be inferred that the portion of the daily oxalate which arises from metabolism of ascorbate is formed and excreted at a constant rate. Further in 6 men of diverse body weight and degree of fatness, it was found that the ascorbate utilization, as expressed in terms of C^{14} oxalate excretion, occurred at a rate of 0.207 mg/day/kg of fat-free body weight. Rarely, if ever, do adult males exceed 90 kg in lean body mass. Therefore, 18 mg/day intake would match the greatest quantity of ascorbate metabolized by the largest healthy man.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-20 Studies on the Metabolism of Pentoses

Study No. 2 Metabolism of D-glucuronolactone-6-C¹⁴
and D-glucuronic-6-C¹⁴ Acid in Man

Description:

Since this is a final report and the material has been published, only a discussion and summary of the work will be presented here.

Discussion:

Studies of body composition and the use of C¹⁴ isotopes have here resulted in a method for stating the actual utilization of ascorbic acid by healthy men. After ingesting a single 20 μ c quantity of D-glucuronolactone-6-C¹⁴, the urine of healthy men contains C¹⁴ activity which can be isolated and found in the highly purified phenylhydrazine derivative of ascorbic acid. Such activity is not found following the ingestion of D-glucuronic-6-C¹⁴ acid, indicating that the above lactone, but not its acid, can function as a source of ascorbic acid.

In subjects who ingest 20 μ c of L-ascorbic-1-C¹⁴ acid, the daily urinary oxalate arising from metabolism of the labeled ascorbate is subsequently excreted as a constant proportion of the total C¹⁴ activity remaining in the body. Thus, it can be inferred that the portion of the daily oxalate which arises from metabolism of ascorbate is formed and excreted at a constant rate. Note, since Crawhall et al.

Sub-task No. 2-20, Study No. 2

showed that about 40% of the daily oxalate came from breakdown of glycine and here it is shown that another 50% comes from ascorbate, it is possible to speculate that nearly all of the urinary oxalate comes from the metabolism of glycine and ascorbate.

The ingestion of a single, comparatively large 0.5 gm of quantity of "cold" ascorbic acid or its precursors by a subject whose body ascorbic acid pool has been previously labeled, as described above, results in increased excretion of C^{14} ascorbate of lowered specific activity. These effects are transitory in that within 2 days the total ascorbate excretion returns to previous levels and the ascorbate specific activity is lower than it was prior to dilution of the body ascorbate pool.

Simultaneously, the total activity and the specific activity of the oxalate decreases, but the proportionality of the total oxalate activity to the specific activity of the ascorbate remains the same. From these effects, it can be inferred that the utilization breakdown of ascorbic acid in the body occurs at a constant rate irrespective of an increased rate of supply of ascorbate to the body.

Further, in 6 men of diverse body weight and degree of fatness, it was found that the ascorbate utilization, as expressed in terms of C^{14} oxalate excretion, occurred at a rate of 0.207 mg per day per kilogram of fat-free body weight. Rarely, if ever, do adult males exceed 90 kg in lean body mass. Therefore, 18 mg per day

Sub-task No. 2-20, Study No. 2

intake would match the greatest quantity of ascorbate metabolized by the largest healthy man.

Summary:

Results of studies with healthy men revealed that close to one-fourth of D-glucuronolactone-6-C¹⁴ was converted to L-ascorbic acid, whereas, on the other hand, no activity could be detected in the ascorbate derivative isolated from the urine of subjects receiving D-glucuronic-6-C¹⁴ acid.

One-half of the urinary oxalate arises from the breakdown of ascorbic acid and is excreted at a constant rate.

The size of the ascorbic acid pool and its rate of utilization was directly related to the size of the fat-free body weight.

List of Publications:

1. Baker, E. M., E. L. Bierman and I. C. Plough. Factors influencing urinary excretion of ketopentoses in normal men. *Metabolism* 9: 478, 1960.

2. Baker, E. M., I. C. Plough and E. L. Bierman. Effect of D-glucuronic acid and D-glucuronolactone on ascorbic acid levels in blood and urine of man and dog. *Am. J. Clin. Nutr.* 8: 369, 1960.

3. Baker, E. M., H. E. Sauberlich and S. J. Wolfskill. Metabolism of D-glucuronolactone-6-C¹⁴ and D-glucuronic-6-C¹⁴ acid in man. *Fed. Proc.* 20: 85, 1961.

4. Baker, E. M., H. E. Sauberlich, S. J. Wolfskill, W. T. Wallace and E. E. Dean. Tracer studies of vitamin C utilization in men; metabolism of D-glucuronolactone-6-C¹⁴, D-glucuronic-6-C¹⁴ acid and L-ascorbic-1-C¹⁴ acid. *Proc. Soc. Exp. Biol. Med.* 109: 737, 1962.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-21 Analytical Chemistry Methodology and its
Application to Medical and Nutritional Research

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-21 Analytical Chemistry Methodology and its
Application to Medical and Nutritional Research

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

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Increasing hydrolysis time, solvent washes and filter density resulted in fecal cellulose values with a $\pm 2\%$ deviation of replicates. The free ammonia in protein hydrolysates for intravenous feeding may be removed without disturbing ionic balance by use of a cupric chelating resin. Diffusion of air into the oil-nitrogen system for ninhydrin in an automatic amino acid analyzer with its resultant change in ninhydrin reactivity may be corrected by an additional two bottle oil-nitrogen complex. Several infants with disorders of the central nervous system exhibited deficiencies of glutamic acid, proline, tyrosine and phenylalanine in their plasma amino acid levels and deficiencies in glycine, cystine and arginine in their urine levels.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-21 Analytical Chemistry Methodology and its
Application to Medical and Nutritional Research

Description:

To support divisional research, methodology is developed by revision of existing procedures, by a combination of procedures or by direct innovation. As a secondary function, support is provided for the collaborative projects of outside units in the form of specific analytical methods.

Progress:

The procedure of Crampton and Maynard for the determination of cellulose was modified to quantitate the amount of alpha cellulose in fecal samples. Increased hydrolysis time, repeated washings with organic solvents and a dense Gooch asbestos filter pad gave replicates with a deviation of $\pm 2\%$.

Monoethanolamine is an excellent absorptive for carbon dioxide in a micro combustion train and reacts with ninhydrin on heating according to Beer's law.. Levels up to 10 mg of carbon may be assayed spectrophotometrically at 570 millimicrons.

In the quest for a balanced intravenous fat emulsion a protein hydrolysate (8 gm/100 ml) and a 5% dextrose solution were incorporated into cottonseed oil preparations. The subsequent toxicity of this formula was possibly based on the presence of abnormal levels of free ammonia in the protein hydrolysis. Analysis revealed 18.4 to

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22.3 mg of free ammonia per 100 ml of hydrolysate. This ammonia may be removed without upsetting the ionic balance by use of a chelating resin in the cupric form which has a strong affinity for the copper-ammonia complex.

The effect of time on the ninhydrin reagent employed in the Spinco Amino Acid Analyzer showed a progressive decrease in molar concentrations of the individual amino acids, falling to a 12% lower value in three weeks. Diffusion of air into the protective oil baths and resultant changes in ninhydrin reagent can be decreased by installation of an additional two bottle oil-nitrogen system.

A 1960 ICNND survey of the food intake of the military personnel of Thailand revealed one of the most important items in the daily diet to be a fish sauce, used freely as a condiment on rice and other daily dietary staples. Direct analysis showed only minor quantities of the essential amino acids. Unidentifiable ninhydrin reactants account for 0.351% of the alpha amino nitrogen. Of the total nitrogen (2.075%), 0.218% is non-protein nitrogen.

A series of collaborative studies with the Pediatric Clinic of Fitzsimons General Hospital was conducted to determine the extent that normal patterns of blood and urine amino acids are altered in infants with disorders of the central nervous system (i.e., phenylketonuria). Deficiencies of glutamic acid, proline, tyrosine and phenylalanine were observed in the plasma amino acid patterns and reduced levels of glycine, cystine and arginine in the urine.

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In cooperation with the University of Colorado Medical Center, these values were compared with those of normal infants. The limits of a deficiency level were difficult to define, since the range of values in normal patterns was so broad.

Summary and Conclusions:

As listed under "Progress."

List of Publications:

1. Adams, Robert A., Wm. J. Stanmeyer and Richard S. Harding. Antarctic stress and vitamin requirements. J. Dental Med. 17: 36, 1962.
2. Powell, R. C., W. T. Nunes, R. S. Harding and J. B. Vacca. The influence of non-absorbable antibiotics on serum lipids and the excretion of neutral sterols and bile acids. Amer. J. Clin. Nutr. (in press).
3. Harding, R. S. and J. A. Dickerman. A paper electrophoretic study of the effect of sarcoidosis on serum globulin levels. Submission pending case history reports.

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Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-24 Amino Acid Interrelationships
(Study Nos. 1-4 Relationship of Essential to
Nonessential Amino Acids in Nutrition; Influence
of Protein Level on the Serum Protein Components
of the Growing Chick; Amino Acid Requirements in
the Mouse-Influence of Protein Level; Influence
of Type of Carbohydrate on Growth and Serum
Cholesterol Levels in Mice Fed Low- or High-
Protein Diets)

U. S. Army Medical Research and Nutrition Laboratory
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1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X50-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-24 Amino Acid Interrelationships

Study Nos. 1-4 Relationship of Essential to Non-essential Amino Acids in Nutrition; Influence of Protein Level on the Serum Protein Components of the Growing Chick; Amino Acid Requirements in the Mouse - Influence of Protein Level; Influence of Type of Carbohydrate on Growth and Serum Cholesterol Levels in Mice Fed Low- or High-Protein Diets

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: G. A. Leveille, H. E. Sauberlich, J. A. Edelbrock, W. C. Goad and H. J. Schneider

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The casein-amino acid diet developed for the determination of amino acid requirements of mice has been found to be unsatisfactory for the determination of precise requirements. Presumably, this is due to variation in the amino acid composition of the casein employed.

Various dietary carbohydrates were found to have no influence on weight gain or plasma cholesterol levels of mice fed two levels of protein.

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Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-24 Amino Acid Interrelationships

Study Nos. 1-4 Relationship of Essential to Non-essential Amino Acids in Nutrition; Influence of Protein Level on the Serum Protein Components of the Growing Chick; Amino Acid Requirements in the Mouse - Influence of Protein Level; Influence of Type of Carbohydrate on Growth and Serum Cholesterol Levels in Mice Fed Low- or High-Protein Diets

Description:

A knowledge of the amino acid requirements of the mouse would be of value in view of the use of this species in nutritional studies. For this reason, efforts are being directed to the development of a diet suitable for the determination of such requirements.

Other studies have been directed towards determining the influence of various dietary carbohydrates on certain biochemical criteria. Further studies on the influence of protein level and essential vs. nonessential nitrogen on amino acid requirements and the significance of protein reserves are being considered.

Progress:

In the last report, the development of a casein-amino acid diet to be used for the determination of essential amino acid requirements of the mouse was described. The use of this diet in determining the sulfur amino acid requirements of the mouse was also described.

Sub-task No. 2-24, Study Nos. 1-4

Several studies have been initiated in an attempt to determine the requirements of other amino acids. However, presumably because of the variations in the amino acid composition of different lots of casein employed, accurate requirements could not be determined. In order to obviate such difficulties, studies will be initiated to attempt to develop a free amino acid diet which will support maximum growth of weanling mice. Such a diet would enable complete freedom in varying levels of single amino acids and should thereby enable the determination of precise requirement values.

In a study of the influence of dietary carbohydrate on weight gain and plasma cholesterol levels of mice, sucrose, glucose, starch and dextrin were found to be without influence.

Since the last report, no further studies have been conducted on the influence of essential vs. nonessential amino acids on growth of rats or of level of protein on serum protein components of chicks. However, several new studies are presently being planned. The influence of essential and nonessential amino acids on the growth of rats will be studied with free amino acid diets. Such diets will enable a greater manipulation of the various amino acids.

The value of protein reserves will be further studied by attempting to relate antibody formation to dietary and serum protein levels. Because of the possible significance of protein reserves to resistance to infections, such studies are pertinent.

Sub-task No. 2-24, Study Nos. 1-4

Summary and Conclusions:

The studies reported demonstrate that 1) dietary carbohydrates are without influence on weight gain or plasma cholesterol levels of weanling mice; 2) a casein-amino acid diet is unsatisfactory for the determination of amino acid requirements of weanling mice.

List of Publications:

1. Leveille, G. A., H. Fisher and A. S. Feigenbaum. Dietary protein and its effects on serum proteins of the chick. *Annals N.Y. Acad. Sci.* 24: 265, 1961.
2. Leveille, G. A. and H. E. Sauberlich. The influence of dietary protein level on serum protein components and cholesterol in the growing chick. *J. Nutrition* 74: 500, 1961.
3. Leveille, G. A., H. E. Sauberlich and J. W. Shockley. The influence of dietary protein level on the essential amino acid requirement of the weanling rat. *U. S. Army Med. Rsch. & Nutr. Lab. Rpt.* #262, 1 Sept. 1961.
4. *Ibid.* The sulfur amino acid requirement for growth of mice fed two levels of nitrogen. *J. Nutrition* 75: 455, 1961.
5. Leveille, G. A., J. W. Shockley and H. E. Sauberlich. The influence of dietary factors on plasma cholesterol of growing mice. *U. S. Army Med. Rsch. & Nutr. Lab. Rpt.* #265, 11 Jan. 1962.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-25 Lipids and Steroids in Nutrition
(Study Nos. 1-12 Influence of Various Dietary
Factors on Plasma Lipids of the Chick, Mouse,
Rat and Human; the Development of Analytical
Methods for Lipid Studies)

U. S. Army Medical Research and Nutrition Laboratory
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1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-25 Lipids and Steroids in Nutrition

Study Nos. 1-12 Influence of Various Dietary
Factors on Plasma Lipids of the
Chick, Mouse, Rat and Human; the
Development of Analytical Methods
for Lipid Studies

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
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1 July 1961 - 30 June 1962

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In young adult mice, dietary protein and sulfur amino acids have been found to depress plasma and liver cholesterol levels, while these dietary supplements do not appear to influence the cholesterol level of growing mice.

Studies on the influence of dietary fat on cholesterol metabolism in chicks demonstrate that the more highly unsaturated fats increase the degree of absorption of dietary cholesterol and, consequently, increase the plasma and liver cholesterol levels.

Cholic and lithocholic acids have been found to elevate plasma cholesterol levels of animals fed a cholesterol-

Sub-task No. 2-25, Study Nos. 1-12

containing or free diet. The influence of these acids on liver cholesterol levels is presently being studied.

Preliminary data have been obtained on the fatty acid composition of plasma glycerides, cholesterol esters and phospholipids of chicks fed high or low-protein diets with or without added cholesterol. The influence of several amino acids and antibiotics on the plasma cholesterol level of the chick have also been studied.

In growing cholesterol-fed rats, dietary saponin and pectin appear to depress cholesterol by increasing fecal bile acid excretion.

Studies in human subjects have shown dietary protein to be without influence on plasma cholesterol levels. The hypocholesterolemic effect of oral neomycin appears to be a result of the impaired fat absorption which accompanies neomycin administration.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-25 Lipids and Steroids in Nutrition

Study Nos. 1-12 Influence of Various Dietary
Factors on Plasma Lipids of the
Chick, Mouse, Rat and Human; the
Development of Analytical Methods
for Lipid Studies

Description:

The influence of nutritional factors on lipid levels and metabolism are being studied. The apparent significance of lipid metabolism to human health makes such studies of vital importance. Continuous attention is given to the development and implementation of analytical procedures essential for such studies.

Progress:

Methodology

The procedures described in the last report have been implemented successfully and are presently being routinely employed in this laboratory. Presently, efforts are being directed to the application of gas chromatography for the determination of steroids and for isolation of steroids, fatty acids and possibly other compounds.

The adaptation of new procedures for bile acid and vitamin A determinations is presently being considered.

Protein, Sulfur Amino Acids and Plasma Cholesterol in Mice

In contrast to the lack of effect of dietary protein on the plasma cholesterol level of weanling mice previously reported, a

Sub-task No. 2-25, Study Nos. 1-12[©]
hypocholesterolemic effect has been noted in young adult mice.

Results obtained to date show a highly significant decrease in plasma and liver cholesterol levels of mice fed 36% casein as compared to 9%. Mice were fed 9, 18, 27 or 36% casein and the regression of plasma or liver, cholesterol or lipid phosphorus on dietary protein level was found to be statistically significant. This observation has been made whether the animals were fed cholesterol or not. The study has been repeated a number of times and the results reproduced in each instance.

In an initial study, the influence of the high protein levels has been found to be largely attributable to the greater content of sulfur amino acids. Mice were fed 9 or 36% dietary protein from casein. A third group was fed 9% casein with an amount of methionine or cystine equivalent to that supplied by 36% casein; these three diets were fed with and without added cholesterol. The addition of sulfur amino acids to the 9% casein diet resulted in plasma and liver cholesterol levels comparable to those observed in animals fed 36% casein. Similar studies will be initiated to determine the reproducibility of the observed results.

Dietary Factors and Plasma and Liver Lipids of Chicks

As reported last year, sulfur amino acids have been found to have a cholesterol-depressing effect in chicks fed diets containing marginal levels of these acids. However, sulfur amino acids have not been found to exert any hypocholesterolemic effect in animals already receiving levels adequate to support maximum growth. It has, therefore, been concluded from these data that, although the

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sulfur amino acid content of a protein may in part be responsible for the cholesterol-depressing effect of the protein, protein has a specific cholesterol-depressing effect unrelated to its sulfur amino acid content. This cholesterol-depressing effect of protein has also been shown to be unrelated to growth. Other amino acids were studied, namely lysine, arginine and tryptophan, and none were found to influence plasma cholesterol when added to diets containing sufficient quantities of these amino acids to support maximum growth. Lysine supplementation of diets deficient in this amino acid did depress plasma cholesterol; however, this was probably the result of increasing the protein available for growth.

In the last report on this project, it was reported that several antibiotics, including penicillin and neomycin, depressed plasma cholesterol levels of growing chicks; in a second study no such effect was noted. In comparing the results of the two studies, another difference becomes apparent. In the first study the antibiotics stimulated growth while in the second study no growth stimulation was noted. Although further studies will be required to clarify these apparent discrepancies, it seems that a plausible explanation based on the difference in growth might be that a sub-clinical infection which responded to the antibiotics used was present in the first study, but not in the second. Further study is anticipated in this area during the next year.

Studies with growing chicks demonstrate that the more unsaturated fats fed (olive and corn oil) increase cholesterol absorption as

Sub-task No. 2-25, Study Nos. 1-12

compared to animals fed a low-fat diet (0.5%) or a saturated fat (coconut oil). In cholesterol-fed chicks, the level of plasma cholesterol was lower in animals receiving the low-fat diet than in those fed any of the fat-supplemented diets. Of the fat-supplemented groups, the chicks receiving coconut oil had a higher level of plasma cholesterol than did chicks fed olive or corn oil. The level of cholesterol in the liver of chicks fed olive oil was higher than for any other treatment; the chicks fed corn oil had lower liver cholesterol levels, followed by the coconut oil and low-fat groups. The values for aortic cholesterol followed a similar trend. In chicks not fed cholesterol, plasma cholesterol levels were highest in the corn or olive oil-supplemented groups; liver cholesterol values were similar in all groups. The cholesterol: phospholipid (C:P) ratios were found to be similar in α - and β -lipoproteins of chicks fed a cholesterol-free diet. In comparing C:P values for chicks fed diets with and without cholesterol, the ratio increased by one-third (0.58 to 0.77) in the α -lipoprotein fraction and by approximately six-fold in the β -fraction (0.44 to 2.69); this indicates that the cholesterol increases particularly in the β -lipoprotein fraction at a much faster rate than does the phospholipid level. The data obtained also demonstrate that the hypercholesterolemia resulting from cholesterol feeding takes place exclusively in the β -lipoprotein fraction with little or no change in the α -cholesterol level.

Sub-task No. 2-25, Study Nos. 1-12

Because of conflicting reports on the influence of the bile acids, cholic and lithocholic acids, on cholesterol metabolism in the chick, studies were initiated to elucidate their effect. In the preliminary study, lithocholic acid elevated plasma cholesterol levels in chicks fed cholesterol-free and supplemented diets, while cholic acid elevated plasma cholesterol levels of cholesterol-fed chicks only. In a second study, chicks were fed a stock diet for approximately four weeks as compared to one week in the first study; the experimental diets were then fed for four weeks in both experiments. The data of the second study were essentially identical to those of the first except that cholic acid elevated plasma cholesterol in chicks fed a diet free of cholesterol. Since this observation is in contrast to that of the first study, it can be interpreted as indicating an influence of age. In order to overcome any age factor, a third study is in progress in which chicks were fed a stock diet for one week and will be fed the experimental diets for 8 weeks; weekly plasma cholesterol values will be obtained. The liver lipid data have not as yet been completed, but the data available indicate that dietary lithocholic acid cause approximately a two-fold increase in liver weight and an elevation in liver cholesterol in chicks fed a cholesterol-free diet, and in contrast appears to depress liver cholesterol in the cholesterol-fed chick. Lithocholic acid also appears to depress liver fat.

Preliminary data have been obtained on the fatty acid composition of glyceride, cholesterol ester and phospholipid fractions of α - and

Sub-task No. 2-25, Study Nos. 1-12

β -lipoprotein lipids in plasma obtained from chicks made hypercholesterolemic by 1) increasing endogenous (feeding a low-protein diet) or 2) exogenous cholesterol (cholesterol feeding). The data thus far obtained indicate that the hypercholesterolemic response is accompanied by a decrease in linoleic and arachidonic acids and an increase in the level of oleic acid or an increase in the oleic: linoleic + arachidonic ratio. More data are being obtained in order to establish such changes more definitely and to delineate the mechanisms involved.

Pectin, Saponin and Plasma Cholesterol of Rats

Rats fed cholesterol had elevated plasma and liver cholesterol levels in comparison to animals fed a cholesterol-free diet (plasma, 101 vs. 128 mg/100 ml; liver, 4.30 vs. 10.32 mg/gm). The plasma and liver cholesterol levels of the cholesterol-fed rats were depressed by the dietary supplementation of 5% pectin or 0.5% saponin (plasma, 128, 116 and 119 mg/100 ml for control, pectin and saponin groups, respectively; liver, 10.32, 7.49 and 7.47 mg/gm for control, pectin and saponin groups, respectively). The hypocholesterolemic effect appeared to be the result of an increased fecal bile acid excretion (17.93, 23.59 and 25.96 mg of di- and trihydroxycholanolic acids excreted/day for control, pectin and saponin groups, respectively) and not due to an increase in fecal neutral sterol excretion (142, 141 and 132 mg sterol excreted/day for control, pectin and saponin groups, respectively). The possibility exists that pectin and saponin may have depressed fat absorption; however, the neutral

Sub-task No. 2-25, Study Nos. 1-12

sterol data do not support such a conclusion. Other studies are being initiated to further elucidate the mechanism of action of pectin and saponin.

Dietary Protein and Neomycin in Human Subjects

Dietary protein level was found to be without effect on the plasma lipids of young normal adult males. High and low-protein diets (130 and 30 gm/day, respectively) were fed and no significant changes in plasma cholesterol, lipid phosphorus and glycerides were noted.

A study has been completed to determine the mechanism(s) involved in the hypocholesterolemic effect of neomycin. Since a small amount of the administered oral dose is absorbed, it was necessary to determine whether the effect might not be a systemic one. The administration of 200 mg of neomycin per day intramuscularly did not alter plasma lipids. The administration of this same dose orally did not alter plasma lipids but did result in stools which were negative for the microorganisms assayed for, indicating that the cholesterol depression was not mediated through the antibiotic's alteration of the intestinal microflora. The administration of 2 gm of neomycin per day depressed plasma cholesterol and significantly reduced fat digestibility. These data are interpreted to show that the hypocholesterolemia resulting from neomycin administration is mediated through its impairment of lipid absorption.

Sub-task No. 2-25, Study Nos. 1-12

Summary and Conclusions:

The results of the studies reported are summarized as follows:

1. Dietary protein and sulfur amino acids have been observed to depress the plasma and liver cholesterol levels of young adult mice.
2. In growing chicks, dietary protein was found to have a greater plasma cholesterol-depressing effect than could be attributed to its sulfur amino acid content.
3. Arginine, tryptophan and lysine did not depress plasma cholesterol levels of growing chicks.
4. The influence of antibiotics on plasma cholesterol levels of chicks appears to be related to their ability to stimulate growth, probably by suppressing sub-clinical infections.
5. A hypercholesteremic response in the chick appears to be accompanied by decreased levels of linoleate and arachidonate with an accompanying increase in oleate. These changes appear to be most apparent in the cholesterol ester fraction and least so in the phospholipid fraction with intermediate values for the glyceride fraction.
6. Saponin and pectin added to the diet of the cholesterol-fed rat depresses plasma and liver cholesterol levels by increasing bile acid excretion.
7. Dietary lithocholic acid has been observed to have a hypercholesteremic effect in the growing chick. Cholic acid also has a hypercholesteremic effect which appears to be influenced by age. Lithocholic acid appears to cause an increase in liver size.

Sub-task No. 2-25, Study Nos. 1-12

8. Dietary protein has been found to be without effect on the plasma lipid levels of human subjects.

9. Dietary neomycin appears to depress plasma lipids in human subjects by impairing lipid absorption.

List of Publications:

1. Leveille, G. A., J. W. Shockley and H. E. Sauberlich.
Influence of the presence of cholesterol and fatty acids on plasma glyceride determination in the chick. Poultry Sci. 40: 1361, 1961.
2. _____ Influence of dietary factors on plasma lipid relationships in the growing chick. Proc. Soc. Exp. Biol. Med. 108: 313, 1961.
3. _____ Lipid distribution in lipoproteins separated by polyanion precipitation. Ibid. 108: 544, 1961.
4. _____ Lipid content of chick erythrocytes and plasma. Ibid. 109: 345, 1962.
5. _____ The influence of dietary factors on plasma cholesterol of growing mice. U. S. Army Med. Rsch. & Nutr. Lab. Rpt. #265, 11 Jan. 1962.
6. _____ Influence of dietary protein level and amino acids on plasma cholesterol of the growing chick. J. Nutrition 76: 321, 1962.
7. Leveille, G. A., H. E. Sauberlich, R. C. Powell and W. T. Nunes. Influence of dietary protein on plasma lipids of human subjects. J. Clin. Invest. 41: 1007, 1962.

Sub-task No. 2-25, Study Nos. 1-12

8. Leveille, G. A., R. C. Powell, H. E. Sauberlich and
W. T. Nunes. Effect of orally and parenterally administered
neomycin on plasma lipids of human subjects. (In preparation)

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-26 Thiamine Metabolism (Study No. 1
An Investigation of the Biochemical Changes Induced
in Humans by a Deficiency of Thiamine)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry and Metabolic
Divisions

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-26 Thiamine Metabolism

Study No. 1 An Investigation of the Biochemical
Changes Induced in Humans by a
Deficiency of Thiamine

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Z. Z. Ziporin, W. T. Nunes, R. C. Powell and H. E. Sauberlich

RCS MEDDH-288

Unclassified

Previous studies reported in the literature have shown disturbances in enzyme function in thiamine deficiency. These biochemical changes do not lend themselves readily to rapid and reliable methods for the assessment of thiamine intake as well as nutritional status. Recent work has shown that red blood cell transketolase may provide a reliable index of thiamine status. Further, new methodology has made it possible to measure the metabolites of thiamine in urine. A study using human volunteer test subjects was undertaken to measure red blood cell transketolase and urinary metabolites during control, depletion and low-level repletion periods.

This study was initially reported in the 1960-61 progress report. The data have been further evaluated to yield several conclusions. Urinary output of metabolites was decreased during the depletion,

Sub-task No. 2-26, Study No. 1

but not to the same extent as intact thiamine which dropped to insignificant amounts during the first week. Transketolase values coincide with depletion and repletion periods, decreasing during the former and increasing during the latter treatments. Creatinine blood cholesterol, free fatty acids and phospholipids showed no change as a result of the thiamine deficiency.

BODY OF REPORT

Project No. 6X50-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Subtask No. 2-26 Thiamine Metabolism

Study No. 1 An Investigation of the Biochemical
Changes Induced in Humans by a
Deficiency of Thiamine

Description:

With the development of a method for measuring the excretion of thiamine metabolites as well as intact thiamine in the urine, a new parameter for the estimation of nutritional status was available. The test with normal human subjects was designed to assess the validity of this parameter and to further investigate the metabolism of thiamine.

Progress:

Data of the previous study have been further evaluated and a manuscript is being prepared for publication. Additional investigations on thiamine metabolism and requirements of the human will be dependent upon the program of the Metabolic Division and the availability of volunteer test subjects.

Summary and Conclusions:

The further evaluation of the data of this study has resulted in the following conclusions: The excretion of intact thiamine is not a reliable index of thiamine deficiency or sufficiency since

Sub-task No. 2-26, Study No. 1

this reflects only the recent levels of intake. As soon as the intake of thiamine decreased, there was an abrupt reduction in the excretion of intact thiamine. Metabolite excretion continued at rather high levels even when signs of deficiency appeared. Lack of thiamine appeared to have no effect on creatinine excretion or blood cholesterol.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-27 The Biochemical Basis for the
Chemotherapeutic Action of Isoniazid (Study No. 2
Influence of INH on B₆ Requirement and on Urinary
Oxalate Excretion in Tubercular Patients)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-27 The Biochemical Basis for the Chemotherapeutic Action of Isoniazid

Study No. 2 Influence of INH on B₆ Requirement
and on Urinary Oxalate Excretion in
Tubercular Patients

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962 .

Authors: E. M. Baker, H. E. Sauberlich, W. T. Wallace and S. J.
Wolfskill .

RCS MEDDH-288

Unclassified

In a 6-month study on a TB patient from this hospital who was receiving INH and PAS, it was noted that even though his 3-hydroxykynurenine excretion values were elevated following tryptophan load his xanthurenic acid excretion values did not significantly change during the period that he was off his B₆ supplement and on INH and PAS treatment. Further, using L-ascorbic-1-C¹⁴ acid to study this patient's ascorbate metabolism, it was found that there was no change in the patient's ascorbic acid pool size or utilization whether on or off INH. Further, there was no change in his oxalate excretion whether on or off INH.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-27 The Biochemical Basis for the Chemotherapeutic Action of Isoniazid

Study No. 2 Influence of INH on B₆ Requirement and on Urinary Oxalate Excretion in Tubercular Patients

Description:

The objectives of this experiment are as follows:

1. Study the tryptophan metabolism in patients who are receiving INH therapy.
2. Study the effect of INH on ascorbic acid metabolism in TB patients to determine whether or not there is an increased utilization of ascorbate.
3. Study the effect of INH on urinary oxalate excretion in TB patients.

Progress:

In a 6-month study on a TB patient from this hospital who was receiving INH and PAS, it was noted that his 3-hydroxykynurenine excretion was markedly elevated even before his 100 mg/day B₆ supplement was taken away. When his B₆ was taken away, no significant changes occurred in his xanthurenic acid excretion following tryptophan load, while on the other hand the 3-hydroxykynurenine excretion became markedly elevated. Blood amino acid levels were increased during this same period while the urine amino acid pattern

Sub-task No. 2-27, Study No. 2

demonstrated a significant increase in the glycine and alanine excretion only. The 3-hydroxykynurenine excretion following tryptophan load still did not return to normal after a 3-week period when the patient was off his INH and PAS therapy but allowed 100 mg B₆/day. The 3-hydroxykynurenine excretion values following tryptophan load during this 3-week period fell exponentially even though the control values were normal (i.e., 5-20 mg/day) indicating the possibility of a true enzymatic block existing. When the patient was put back on INH and PAS therapy, even with his 100 mg/day B₆ supplement, the 3-hydroxykynurenine level of excretion immediately rose following tryptophan load.

Using L-ascorbic-1-C¹⁴ acid to study this patient's ascorbate metabolism, it was found that there was no change in the patient's ascorbic acid pool size or utilization whether on or off INH therapy. Further, there was no change in his oxalate excretion whether on or off INH therapy.

Summary and Conclusions:

3-hydroxykynurenine excretion was markedly increased in the patient studied here even before B₆ was taken away. No significant xanthurenic acid excretion, following tryptophan load, occurred during the period the patient was off B₆ supplement while, on the other hand, the 3-hydroxykynurenine excretion became markedly elevated.

Sub-task No. 2-27, Study No. 2

The 3-hydroxykynurenine excretion still did not return to normal after a 3-week period off INH. The 3-hydroxykynurenine excretion values, following tryptophan load, fell exponentially even though the control values were normal (i.e., 5-20 mg/day) indicating the possibility of an enzymatic block in tryptophan metabolism. When the patient was put back on INH with B₆ supplement, the 3-hydroxykynurenine excretion following tryptophan load immediately rose.

There was no change in the patient's ascorbic acid pool size or utilization whether on or off INH. Further, there was no change in his oxalate excretion whether on or off INH therapy.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. C2 - Metabolism and Nutrition

Sub-task No. 2-27 The Biochemical Basis for the
Chemotherapeutic Action of Isoniazid (Study No. 4
High Isoniazid-Blood Ammonia)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

March 1962 - 30 June 1962

Z. Z. Ziporin, Ph.D.
W. C. Morse, Lt. Col., MSC
and
C. S. Christianson, Col., MC

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ABSTRACT

Project No. 6X50-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-27 The Biochemical Basis for the Chemotherapeutic Action of Isoniazid

Study No. 4 High Isoniazid-Blood Ammonia

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

March 1962 - 30 June 1962

Authors: Z. Z. Ziporin, W. C. Morse and C. S. Christianson

RCS MEDDH-288

Unclassified

Previous work in this laboratory has shown that patients receiving isoniazid have elevated blood ammonia values. It was further shown that the elevated ammonia appeared only when the isoniazid was administered and was not present when PAS, SM or B₆ were given without isoniazid. Thus, it was felt that the toxic psychosis which may appear in patients given high doses of isoniazid might be due to an elevation of blood ammonia. These patients exhibit symptoms of confusion, disorientation and hallucination. The study was designed to test whether patients exhibiting these symptoms do have high blood ammonia values. Further, it will be determined whether lowering blood ammonia by glutamic acid or arginine will help the patient to better tolerate higher doses of isoniazid.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-27 The Biochemical Basis for the Chemotherapeutic Action of Isoniazid

Study No. 4 High Isoniazid-Blood Ammonia

Description:

To determine whether patients with toxic psychosis, receiving isoniazid, have elevated blood ammonia values, and whether the reduction of the blood ammonia would result in curing the toxic psychosis and the intolerance for the drug.

Progress:

The Pulmonary Disease Service at Fitzsimons General Hospital is now screening patients for the study. As soon as they are available, the study will be under way.

Summary and Conclusions:

None at this stage of the investigation.

List of Publications:

None.

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Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions (Study No. 4 Factors Affecting the Absorption of Minerals from the Intestinal Tract)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

February 1962 - 30 June 1962

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S. Zolyomi, Sp5
and
K. E. Kinnamon, Capt., VC

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 4 Factors Affecting the Absorption of Minerals from the Intestinal Tract)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

February 1962 - 30 June 1962

Authors: Z. Z. Ziporin, P. P. Waring, S. Zolyomi and K. E. Kinnamon

RCS MEDDH-288

Unclassified

Problems associated with radioactive fallout have been resolved into two main categories: (1) Those relating to radiation injury as a result of external irradiation from radionuclides released by the bomb blast, and (2) those relating to internal irradiation produced by the long-lived radioisotopes which have been shown to be "bone seekers." From the biological viewpoint, the radioactive strontium isotopes, Sr^{90} and Sr^{89} , with half-lives of 28 years and 53 days, respectively, are the most important substances among the fission products.

Previous work has attempted to remove the isotope after it has been deposited in bone. Because of the nature of skeletal dynamics, one might expect a greater chance of success immediately after the incorporation of strontium in bone, with diminishing

Sub-task No. 2-28, Study No. 4

chance for success as time progresses. However, efforts to remove the isotope from bone, once it has been deposited, have met with little success. Our work will probe the conditions in the intestinal tract which would reduce the adsorption of strontium across the intestinal wall.

BODY OF REPORT

Project No. 6X6C-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 4 Factors Affecting the Absorption
of Minerals from the Intestinal Tract)

Description:

Diets of varying compositions will be offered weanling rats at specified intervals during the day. At the end of these feeding periods, doses of Sr^{85} will be administered by stomach tube. Retention of the isotope will be measured by counting Sr^{85} in urine, feces and carcass. This will be correlated with diet composition.

Progress:

Clearance for the use of Sr^{85} has recently been obtained. The studies will be undertaken shortly.

Summary and Conclusions:

None at this time.

List of Publications:

None at this time.

ANNUAL PROGRESS REPORT

Project No. 6X6C-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions (Study No. 5 The Effect of Hepatic Injury on Zinc Metabolism)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 March 1962 - 30 June 1962

G. E. Bunce, Capt., MSC
and
P. G. Reeves, 1st Lt., MSC

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 5 The Effect of Hepatic Injury on
Zinc Metabolism)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 March 1962 - 30 June 1962

Authors: G. E. Bunce, and P. G. Reeves

RCS MEDDH-288

Unclassified

Recent reports of clinical studies of patients with Laennec's cirrhosis have mentioned zincuria and low levels of serum and liver zinc as features of this condition. The authors noted that administration of zinc supplements as low as 15 mg per day was followed by improvement as determined by an increase in serum zinc and a decrease in urinary zinc and in retention of BSP.

This study is designed to investigate zinc metabolism during the course of liver injury induced by hepatotoxic agents. In the first experiment, the effect of injections of carbontetrachloride on liver, serum, and urinary zinc and on liver pyridine nucleotides will be determined using the rat as the experimental animal. The value of increased zinc intake as an ameliorative treatment will also be investigated. Subsequent trials are planned in which liver damage will be induced with other types of noxious agents and by nutritional deficiencies.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 5 The Effect of Hepatic Injury on
Zinc Metabolism)

Description:

The purpose of this study is to investigate the possible role of zinc as an ameliorative factor in hepatic injury caused by various agents.

Progress:

Twenty-four adult rats were divided into two groups and fed a semi-purified diet containing either 100 or 1,000 μ gm zinc per 100 gm of diet. Half of the animals in each group were given subcutaneously injections of carbontechachloride in amounts of 0.1 cc per 100 grams body weight every week for five weeks. Total fecal and urine collections were made during the second, fourth and sixth weeks at which time the animals were sacrificed. The liver tissue will be analyzed for total pyridine nucleotides, total zinc, % nitrogen and % fat. The urine will be analyzed for zinc and N'-methylnicotinamide. Total fecal and serum zinc will also be determined. In addition, the liver and kidney will be evaluated with histopathological techniques. No results are yet available for discussion.

Summary and Conclusions: None

List of Publications: None

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Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-29 Pyridoxine Metabolism (Study No. 4
Glycine Metabolism in B₆ Deficient Adult Rats)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

E. M. Baker, Maj., MSC
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-29 Pyridoxine Metabolism

(Study No. 4 Glycine Metabolism in B₆ Deficient
Adult Rats)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: E. M. Baker, E. E. Sauterlich, W. T. Wallace, and
S. J. Wolfskill

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Unclassified

In studies on B₆ deficient rats it was noted that the urinary oxalate excretion was increased. Further B₆ deficient rats given isoniazid (INH) in their diet demonstrated a marked increase in the excretion of urinary oxalate. The xanthurenic acid excretion in these animals showed an increase in the B₆ deficient, but a decrease in the B₆ deficient +INH groups. Simultaneously, the B₆ deficient +INH group demonstrated a marked increase in 3-hydroxykynurenine excretion. Using C¹⁴ labeled glycine it was found that the conversion of glycine to oxalate increased in the B₆ deficient rats, but decreased in B₆ deficient rats who were fed INH in their diets. When the ascorbic acid conversion to oxalate was studied in these same groups of animals using L-ascorbic-1-C¹⁴ acid it was found that the ascorbate to oxalate conversion decreased

Sub-task No. 2-29, Study No.4

in the B₆ deficient rats and increased in the B₆ deficient animals given INE. It appears, therefore, that the effect of INE on glycine and ascorbate metabolism is separate and specific from dietary B₆ deficiency.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-29 Pyridoxine Metabolism

(Study No. 4 Glycine Metabolism in B₆ Deficient Adult Rats)

Description:

The objectives of this experiment were as follows:

To study the oxalic acid, xanthurenic acid and 3-hydroxy-kynurenine excretion in normal and B₆ deficient rats.

To study the effects of isoniazid (INH) on C¹⁴ labeled glycine and oxalate formation and excretion in B₆ deficient rats.

To study the metabolism of L-ascorbic-1-C¹⁴ acid in B₆ deficient rats in terms of oxalate formation and excretion.

Last, to study the blood and urinary amino acid patterns on INH treated animals.

Progress:

In adult rats that were fed a B₆ deficient diet, the urinary oxalate and xanthurenic acid excretion increased as compared with the control animals. Further, rats fed the same B₆ deficient diet, but supplemented with isoniazid (INH) added to the diet in the amount of 1 gm/kg., demonstrated a 4-fold increase in urinary oxalate excretion as compared with the control animals. It is of interest to note that these same animals (B₆ deficient +INH) showed a marked elevation in urinary 3-hydroxykynurenine excretion while, at the same time, their urinary xanthurenic acid excretion was markedly decreased.

Sub-task No. 2-29, Study No. 4

In studies using glycine- C^{14} to study the conversion of glycine to C^{14} labeled oxalate and the urinary excretion thereof, it was demonstrated that the conversion of glycine to oxalate was increased in B_6 deficient rats. In contrast to this, B_6 deficient rats that were given L-ascorbic- C^{14} acid demonstrated a marked decrease of conversion and excretion of the L-ascorbic acid as oxalate. Pyridoxine deficient rats that were supplemented with INE, on the other hand, demonstrated a marked decrease in the conversion of the C^{14} labeled glycine to oxalate. Conversely, these same animals demonstrated a marked increase in the conversion and excretion of the C^{14} labeled ascorbate to oxalate. The same effects (i.e. decrease in conversion of C^{14} labeled glycine to excreted oxalate and an increase in the conversion of C^{14} labeled ascorbate to excreted oxalate) were noted in the B_6 supplemented +INE animals indicating that the effect of INE on glycine and ascorbate metabolism is separate and specific from dietary B_6 deficiency.

In the B_6 deficient animals, the blood amino acid patterns indicated a slight increase in glycine, asparagine and glutamine values. In the B_6 deficient +INE as well as the B_6 supplemented +INE groups of animals, the values for the following amino acids were markedly elevated: Threonine, serine, glutamic acid, glycine, alanine, leucine, tyrosine, phenylalanine, asparagine, glutamine and taurine.

The urinary amino acid patterns indicated a significant increased excretion only in glycine and alanine in the B_6 deficient, B_6 deficient +INE and B_6 supplemented +INE groups of animals.

Sub-task No. 2-29, Study No. 4

Summary and Conclusions:

The urinary oxalate increased in B₆ deficient rats and markedly increased in B₆ deficient rats given INH in their diet. The xanthurenic acid excretion in these animals showed an increase in the B₆ deficient, but a decrease in the B₆ deficient +INH groups. Simultaneously, the B₆ deficient +INH group demonstrated a marked increase in 3-hydroxykynurenine excretion.

Glycine conversion to oxalate increased in B₆ deficiency in rats, but decreased in B₆ deficient rats that were given INH, while at the same time the ascorbate to oxalate conversion decreased in B₆ deficiency and increased in B₆ deficient animals given INH. It appears, therefore, that the effect of INH on glycine and ascorbate metabolism is separate and specific from dietary B₆ deficiency.

The blood amino acid patterns indicated markedly increased values for 11 amino acids in the B₆ deficient +INH and B₆ supplemented +INH groups of rats as compared to only slightly increased glycine, asparagine and glutamine values for the B₆ deficient animals. The urine amino acid patterns indicated a significant increased excretion only in glycine and alanine in the B₆ deficient, B₆ deficient +INH and B₆ supplemented +INH groups of animals.

List of Publications:

Baker, E. M., E. E. Seuberlich, W. T. Wallace and S. J. Wolfskill.
The Effect of INH on glycine and ascorbic acid metabolism in normal and B₆ deficient rats (in preparation).

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 2-30 Studies on the Metabolism of
Glycine-1-C¹⁴ and Glycine-2-C¹⁴ in Man

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

E. M. Baker, Maj., MSC
H. E. Sauberlich, Ph.D.
W. T. Wallace, Sp4
and
S. J. Wolfskill, Sp4

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-30 Studies on the Metabolism of Glycine-1-C¹⁴ and Glycine-2-C¹⁴ in Man

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: E. M. Baker, H. E. Sauberlich, W. T. Wallace, and S. J. Wolfskill

RCS MEDDH-288

Unclassified

Methods and protocols have been devised to study both glycine-1-C¹⁴ and glycine-2-C¹⁴ metabolism in humans to determine whether or not the glycine C¹⁴ is partially converted to and excreted as oxalate at a constant rate per day as well as the amount of activity per day excreted as urinary oxalate. Further, the expired C¹⁴O₂ will be measured in a vibrating reed electrometer to determine the amount of decarboxylation of C¹⁴ labeled glycine in man.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 2-30 Studies on the Metabolism of Glycine-1-C¹⁴ and Glycine-2-C¹⁴ in Man

Description:

The recent work of Crawhall et al. using glycine-1-C¹³ has demonstrated that this isotope was diluted about $2\frac{1}{2}$ times during the conversion of glycine from the "first metabolic pool" to oxalate, indicating that about 40% of the urinary oxalate was derived from glycine during this period.

It has been demonstrated in this laboratory that 50% of the urinary oxalate was derived from L-ascorbic-1-C¹⁴ acid and was excreted at a constant rate per day. Therefore, it is of interest to study both glycine-1-C¹⁴ and glycine-2-C¹⁴ metabolism in humans to determine (1) whether or not the glycine C¹⁴ is partially converted to and excreted as oxalate at a constant rate per day as well as (2) the amount per day excreted as urinary oxalate.

Further, the expired C¹⁴O₂ will be measured in a vibrating reed electrometer to determine the amount and extent of decarboxylation of the C¹⁴ labeled glycine in man. It should be noted that this has been done with humans using glycine-2-C¹⁴ but not with glycine-1-C¹⁴.

Progress:

Four subjects have been labeled with glycine-1-C¹⁴ and at an interval of 90 days later with glycine-2-C¹⁴. The expired C¹⁴O₂

Sub-task No. 2-30

has been measured in all cases. Further urine samples were collected every third day following the labeling of each subject. Quantitative oxalate determinations have been made on each 24-hour urine sample. At present, the total urine C^{14} activity and the specific activity of each isolated oxalate sample is being counted. All data should be completed by the end of June 1962.

Summary and Conclusions:

None

List of Publications:

None

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-11 Human Pyridoxine Requirements and the Inter-relationships of Pyridoxine to the Dietary Macronutrients

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic and Chemistry Divisions

1 July 1961 - 30 June 1962

John E. Canham, Lt. Col, MC
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-11 Human Pyridoxine Requirements and the Inter-relationships of Pyridoxine to the Dietary Macronutrients

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: J. E. Canham, H. E. Sauberlich, W. T. Nunes, E. M. Baker
and M. E. McDowell

RCS MEDDH-288

Unclassified

Previous studies were conducted in this laboratory to determine the minimal human daily requirement for Vitamin B₆. Though the general requirement for the Vitamin was ascertained, the precise requirement was not determined. Results suggested that the minimal daily requirement of Vitamin B₆ was influenced by the dietary level of protein. No further human studies have been carried out since October 1960. A study, which is to commence on 1 August 1962, has been devised to study in specific detail the minimal human daily requirement for Vitamin B₆, the relationship of this requirement to the level of dietary protein intake, and the biochemical changes induced by Vitamin B₆ depletion.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-11 Human Pyridoxine Requirements and the Inter-relationships of Pyridoxine to the Dietary Macronutrients

Description:

The most recent study (done Sept-Oct 1960) was designed to provide additional information regarding the minimal daily requirement of Vitamin B₆ in relation to the protein intake.

Progress:

No further work has been done since the previous report. A new project is about to commence to better ascertain the precise daily requirements of Vitamin B₆ and the biochemical abnormalities induced by early Vitamin B₆ deficiency.

Summary and Conclusions:

No additional work has been done during the past year, but a new study is planned to commence on 1 August 1962.

List of Publications: None

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-13 Long Term Feeding Study of Irradiated Food

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-13 Long Term Feeding Study of Irradiated Food

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

RCS MEDDH-288

Unclassified

No further work has been done on this project
in the past year. Long term feeding studies in
humans await further evaluation of animal studies
currently in progress.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-13 Long Term Feeding Study of Irradiated Food

Description:

Seven separate short term (15 days) human feeding studies on selected irradiated food items have been conducted previously in this laboratory in order to evaluate acceptability and possible toxicity. Results have been reported previously. Long term feeding studies are planned.

Progress:

No further work has been done during the past year. Long term feeding studies in humans await further evaluation of animal studies currently in progress.

Summary and Conclusions:

No additional work has been done during the past year. A long term study is planned, but unscheduled.

List of Publications:

No new publications during this year.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-15 Algae as a Food Source for Humans

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic Division

1 July 1961 - 30 June 1962

Marion E. McDowell, Lt. Col, MC
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Richard C. Powell, Capt, MC
Elizabeth M. Nevels, Maj, AMSC
Jacqueline H. Sellars, Maj, ANC
Norman F. Witt, Ph.D.

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-15 Algae as a Food Source for Humans

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Marion E. McDowell, Richard C. Powell, Elizabeth M.
Nevels, Jacqueline H. Sellars, and Norman F. Witt

RCS MEDDH-288

Unclassified

The acceptability, digestibility, and acute toxic effects of algae when fed as a food supplement to four volunteers were studied in 1959. No further studies have been conducted during the past year but future studies are contemplated when a sufficient quantity of an improved microalgae becomes available. The paper describing the initial study was published in the J. of Nutrition, 75:1, Sept 1961.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-15 Algae as a Food Source for Humans

Description:

This study was designed to evaluate the use of microalgae as a possible food source for humans with emphasis on acceptability, digestibility, and possible toxic effects.

Progress:

The initial study was carried out on the Metabolic ward in 1959. Although no further studies were performed during the past year, we feel the results are encouraging and hope to complete additional studies when more microalgae is available. The paper describing the initial study was published in the J. of Nutrition, 75:1, Sept. 1961.

Summary and Conclusions:

Further studies on the acceptability and digestibility of microalgae are anticipated when more algae is made available.

List of Publications:

1. McDowell, M. E., Powell, R. C., Nevels, E. M., Sellars, J. H., and Witt, N. F.: Algae Feeding in Humans: Acceptability, Digestibility, and Toxicity. Fed. Proc., 19:319, 1960 (abstract).
2. Powell, R. C., Nevels, E. M., and McDowell, M. E.: Algae Feeding in Humans. J. of Nutrition, 75:1, Sept 1961.

FINAL REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study No. 1
Serum Lipid Studies in Familial Hypercholesterolemic
Xanthomatosis)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic, Chemistry, and Physiology
Divisions

1 July 1961 - 30 June 1962

Richard C. Powell, M.D.
and
Joseph B. Vacca, M.D.

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans
Study No. 1 Serum Lipid Studies in Familial
Hypercholesterolemic Xanthomatosis

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: R. C. Powell and J. B. Vacca

RCS MEDDH-288

Unclassified

No further work on this Study No. 1 is planned.
The results of the study were summarized in last year's
Annual Report and have now been published in the
Am. J. Med., 31:828, Nov 1961.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans
Study No. 1 Serum Lipid Studies in Familial
Hypercholesterolemic Xanthomatosis

Description:

The effects of dietary fat alterations and of a cholesterol synthesis inhibitor on serum lipids in a patient (Fitzsimons General Hospital) with familial hypercholesterolemic xanthomatosis were studied.

Progress:

This study was conducted in cooperation with the Department of Medicine, Fitzsimons General Hospital, between September 1959 and April 1960. The report has been published in the Am. J. Med., 31:828, Nov 1961 and was summarized in last year's Annual Report.

Summary and Conclusions:

No further work is anticipated on Study No. 1.

List of Publications:

Powell, R. C. and Vacca, J. B.: Serum lipid studies in familial hypercholesterolemic xanthomatosis. Am. J. Med., 31:828, Nov 1961.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study No. 2,
Part I The Effects of Various Diets and Drugs on
Serum Lipid Partition and Sterol and Lipid Balance
in Humans)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic, Chemistry and Microbiology
Divisions

1 July 1961 - 30 June 1962

Richard C. Powell, M.D.
and
William T. Nunes, Maj, MC
Richard S. Harding, B.S.
Joseph B. Vacca, M.D.
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans
Study No. 2, Part I The Effects of Various
Diets and Drugs on Serum Lipid Partition and
Sterol and Lipid Balance in Humans

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: R. C. Powell, W. T. Nunes, R. S. Harding, J. B. Vacca,
E. M. Nevels, and H. E. Sauberlich

RCS MEDDH-288

Unclassified

The following abstract is repeated from last year's Annual
Progress Report:

The influence of nonabsorbable antibiotics on serum lipids
and the excretion of sterols and bile acids was studied. Eight
volunteers were placed on a constant diet. After a suitable
control period, neomycin or a combination of polymyxin and
bacitracin was administered orally for 24 days, and this was
followed by a 12-day recovery period. The results indicated
that either antibiotic regimen was capable of lowering serum
cholesterol and lipid phosphorus; and either regimen increased
fecal bile acid and sterol excretion. Neomycin, however, pro-
duced a more dramatic change.

Editing of the final manuscript has now been completed and
the report is in press in the American Journal of Clinical
Nutrition.

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BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition ®

Sub-task No. 3-18 Lipid Metabolism Studies in Humans
Study No. 2, Part I The Effects of Various
Diets and Drugs on Serum Lipid Partition and
Sterol and Lipid Balance in Humans

Description:

This study was designed to evaluate the influence of non-absorbable antibiotics on serum lipids and the fecal excretion of bile acids and sterols.

Progress:

This study was carried out on the Metabolic ward in April, May, and June 1960. The data have been assembled and a report has been accepted for publication in the American Journal of Clinical Nutrition (vide infra).

Summary and Conclusions:

The results of this study were summarized in last year's Annual Progress Report and will soon be published in the American Journal of Clinical Nutrition.

List of Publications:

Powell, R. C., Nunes, W. T., Harding, R. S., Vacca, J. B.:
The influence of nonabsorbable antibiotics on serum lipids and the excretion of neutral sterols and bile acids (in press).

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study
No. 2, Part II The Effect of Antibiotics
on Cholesterol Balance in Humans

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic, Chemistry, and Microbiology
Divisions

1 July 1961 - 30 June 1962

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Richard C. Powell, M.D.
Howerde E. Sauberlich, Ph.D.
William T. Nunes, Maj, MC

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study No. 2,
Part II The Effect of Antibiotics on Cholesterol
Balance in Humans)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: G. A. Leveille, R. C. Powell, H. E. Sauberlich, and
W. T. Nunes

RCS MEDDH-288

Unclassified

A study designed to evaluate the mode of hypocholesterolemic action of neomycin was carried out in six young men by administering low doses of the antibiotic, both by intramuscular injection and by mouth, while observing the serum lipid levels as well as the effect of each treatment on the bacterial flora of the intestine. Parenteral neomycin in small doses had no effect on serum cholesterol. It was shown that despite "sterilization" of the intestine with miniscule oral doses of neomycin, there was no lowering of serum cholesterol. Conversely, when large oral doses were given, "sterilization" occurred in only four of the six subjects. In spite of this, serum cholesterol was reduced in all six subjects. The conclusion drawn is that the hypocholesterolemic effect of neomycin is probably not related to its antibacterial action nor its systemic effect.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study No. 2, Part II: The Effect of Antibiotics on Cholesterol Balance in Humans)

Description:

This study was designed to evaluate the mode of action of nonabsorbable antibiotics on serum lipids and the fecal excretion of bile acids and sterols.

Progress:

In an attempt to more clearly define the mode of hypocholesterolemic action of neomycin, a second project was undertaken during April-July 1961. Six young men were treated with small doses of parenteral neomycin (200 mg/day intramuscularly) as well as with oral neomycin during subsequent balance periods. After a 20-day adjustment period, the subjects received intramuscular neomycin (50 mg every six hours) for a period of 12 days. Following another 20-day control period, oral neomycin (50 mg every six hours) was administered for 12 days. The oral dose was then increased to 0.5 gm every six hours for an additional 12 days. Measurements were carried out on blood, urine, feces, and dietary composites as described in the previous study.

A lowering of serum cholesterol was achieved only during the oral administration of 2.0 gm per day. Microbiological studies showed that, although there was no lowering of serum

cholesterol, "sterilization" of the intestine occurred in four of six subjects taking the lower oral dose of neomycin. While the larger oral dose was administered, "sterilization" occurred in the same four individuals despite the fact that serum cholesterol levels were reduced in all six. Examination of feces for macronutrient content revealed evidence of decreased intestinal absorption during the period in which the higher level of oral neomycin was given.

Conclusion:

From the foregoing data, it seems prudent to conclude that neomycin (as an example of a nonabsorbable antibiotic) exerts a hypocholesterolemic effect through the alteration of intestinal absorption. Specifically, it appears that neomycin interrupts the enterohepatic circulation of bile acids with increased fecal loss of bile acids, increased synthesis of these acids, and increased catabolism of cholesterol. The hypocholesterolemic effect of neomycin is probably not due to its antibacterial action or to its "systemic" effect.

List of Publications:

Report has been prepared and submitted for publication.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study
No. 3 A Comparison of Methods of Obtaining Adipose
Tissue for Analysis of Fatty Acid Composition)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic Division

1 July 1961 - 30 June 1962

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study No. 3
A Comparison of Methods of Obtaining Adipose
Tissue for Analysis of Fatty Acid Composition)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: J. E. Canham, W. T. Nunes, and J. A. Tillotson

RCS MEDDH-288

Unclassified

A study was conducted on human subjects to determine the ideal technique for obtaining subcutaneous depot fat specimens for gas chromatographic analysis. Fat specimens obtained by surgical biopsy and the specimens obtained by needle aspiration disclosed gas chromatographic fatty acid patterns in keeping with those reported in the literature. The patterns of biopsy specimens obtained by a 2-mm dermatologic punch disclosed a higher percentage of saturated fatty acids and shorter chain fatty acids than obtained by the aspiration technique (51.5% C-14 and C-16 fatty acids with 57.1% total saturated fatty acids by punch as compared to 39.8% C-14 and C-16 with 37.8% total saturated fatty acids by aspiration technique) and did not conform to the pattern of the surgical specimen taken at the same depth. When procaine was not used for local

anesthesia prior to the punch biopsy, the fatty acid pattern, though not typical, more closely approximated that previously reported than when procaine was used. Wet sterilization with Wescodyne of the punch prior to use appeared to influence the fat pattern.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-18 Lipid Metabolism Studies in Humans (Study No. 3
A Comparison of Methods of Obtaining Adipose
Tissue for Analysis of Fatty Acid Composition)

Description and Progress:

Gas liquid chromatography permits accurate analysis of fatty acid composition of microquantities of adipose tissue. Hirsch, et al. have described a technique of sampling subcutaneous fat which obviates excisional biopsy and which seems to accurately reflect the fatty acid makeup of adipose tissue when compared to samples obtained by surgery. The technique is eminently simple and painless, and consists in aspiration of subcutaneous fat in the form of droplets into a large (50 ml) syringe through a No. 18 thin-walled needle.

A study was carried out to compare the gas chromatographic analysis of fat specimens obtained by the needle aspiration technique versus specimens obtained by biopsy using a 2-millimeter dermatologic punch. Samples were obtained from four human subjects at three-week intervals for 12 weeks, using both techniques.

Specimens obtained from other volunteers included: punch biopsy specimens obtained with and without procainization of the skin, biopsy specimens obtained with a punch sterilized by Wescodyne and a second, sterilized by heat; a surgical excisional biopsy specimen obtained at the same time as aspiration and

punch biopsy specimens were obtained, and a thick surgical biopsy specimen was obtained while a patient was undergoing surgery.

Gas chromatographic fatty acid patterns of specimens obtained by surgical biopsy and by aspirations were in keeping with those reported in the literature. The patterns of the specimens obtained by punch biopsy disclosed a higher percentage of saturated fatty acids and shorter chain fatty acids than obtained by the aspiration technique (51.5% C-14 and C-16 fatty acids with 57.1% total saturated fatty acids by punch as compared to 39.8% C-14 and C-16 with 37.8% total saturated fatty acids by aspiration technique) and did not conform to the pattern of the surgical specimen taken at the same depth. The fatty acid pattern of punch biopsy specimens obtained without procaine anesthesia, though not typical, more closely approximated the generally accepted normal pattern than when procaine was used. Wet sterilization of the punch in Wescodyne prior to biopsy produced a more "distorted" fatty acid pattern than did the punch sterilized by heat.

Summary and Conclusions:

A study to compare the gas chromatographic fatty acid patterns of subcutaneous fat obtained by various techniques was carried out. Patterns of specimens obtained by aspiration closely approximate those previously reported and those obtained on specimens surgically excised. Patterns of specimens obtained by punch biopsy revealed an increased percentage of the shorter chain fatty acids. The method of sterilization of the punch prior

to its use and the employment of procaine as a local anesthetic were demonstrated to influence the fatty acid pattern of specimens obtained by punch biopsy.

List of Publications:

A report of this study is being written for publication.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-19 The Role of Dietary Partition on the Regulation
of Intermediary Metabolism in Man (Study No. 1)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic and Bioenergetic
Divisions

1 July 1961 - 30 June 1962

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Frank Consolazio
Mary C. Tkacik, Maj, ANC
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-19 The Role of Dietary Partition on the Regulation of Intermediary Metabolism in Man (Study No. 1)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: W. T. Nunes, J. E. Canham, E. M. Nevels, F. Consolazio, M. C. Tkacik, and C. A. Wilber

RCS MEDDH-288

Unclassified

It has been shown that the rate of ingestion of the total daily caloric intake has a pronounced effect on the intermediary metabolism of foodstuffs in various animals, characterized by enhanced lipogenesis. A study has been carried out in four young, healthy men to investigate the effect of periodicity of eating, if any, in the human. Four subjects consumed a 3600 Calorie diet for a 12-week period during which the diet was served in three equal meals, two small meals and one large meal, and in nine small snacks daily. Measurements of energy metabolism and body composition were carried out along with investigation of possible changes in blood lipids and urinary excretion of adrenal steroids. The data are undergoing analysis.

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BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-19 The Role of Dietary Partition on the Regulation of Intermediary Metabolism in Man (Study No. 1)

Description:

A 12-week feeding study was carried out in four young, healthy men in an attempt to elucidate possible metabolic changes induced by varying the rate of calorie consumption.

Progress:

A study of the effect of varied periodicity of eating was carried out in four human subjects over a 12-week period. A diet consisting of 3600 Calories (100 gm protein, 170 gm fat, and 415 gm carbohydrate) was fed in three distinct schedules: During the first two weeks, the subjects consumed the diet in three isocaloric meals. For three weeks, the diet was served in two small snacks (300 Calories each) at breakfast and supper time and a single large meal (3000 Calories) at the noon hour. After one additional week of three equal meals, the subjects were fed the daily caloric ration in nine small snacks for three weeks. The last three weeks of the study were devoted to feeding the diet in three equal portions daily. Activity was supervised at all times and each subject took part in two strenuous exercise periods daily on either a level treadmill or a bicycle ergometer. Measurements included O_2 consumption and CO_2 production throughout the day, body composition by the volumeter, blood lipids (total serum

cholesterol, lipid phosphorus, and glycerides), urinary total nitrogen and creatinine, and urinary 17-ketosteroids and 17-ketogenic steroids. Samples of adipose fat were examined by liquid gas-chromatography for fatty acid pattern. The study has been completed and the available data are being analyzed.

Summary and Conclusions:

The data are currently being analyzed statistically.

List of Publications:

None at this stage of the investigation.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-19 Periodicity of Eating: Its Effect on Inter-
mediary Metabolism in Man (Study No. 2)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic Division.

1 July 1961 - 30 June 1962

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Mary C. Tkacik, Maj, ANC
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-19 Periodicity of Eating: Its Effect on Inter-
mediary Metabolism in Man (Study No. 2)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: W. T. Nunes, J. E. Canham, E. M. Nevels, M. C. Tkacik,
and C. A. Wilber

RCS MEDDH-288

Unclassified

A second study of the effect of periodicity of eating in normal humans was carried out in 11 young men. Subjects were divided into two groups and received a diet containing a plethora of calories. One group received the daily ration in two small snacks and one large meal while the second group consumed the diet in nine small feedings daily. After six weeks, the groups switched dietary pattern, and the diet was fed for an additional six weeks. Measurement of blood lipid and fasting blood glucose was carried out in addition to body composition and analysis of changes in adipose fat. The data are not complete at this time.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-19 Periodicity of Eating: Its Effect on Inter-
mediary Metabolism in Man (Study No. 2)

Description:

In order to further evaluate the effect of varied periodicity of calorie consumption on human metabolism, a second feeding study was carried out in 11 young, healthy men using an excess of calories.

Progress:

A second study of the effect of rate of calorie ingestion was carried out in 11 young men. The study was of 14 weeks duration. The diet consisted of 4050 Calories (100 gm protein, 180 gm fat, and 507.5 gm carbohydrate). The subjects were randomly assigned to one of two groups. After a two-week dietary adjustment phase during which all subjects consumed the diet in three isocaloric meals, one group received the diet in nine small feedings while the second group ate two small feedings (300 Calories, each) and one large meal (3450 Calories). This schedule was continued for six weeks after which the groups switched. The study was continued for an additional six weeks. Observations included: blood lipids (total serum cholesterol, lipid phosphorus, glycerides, and non-esterified fatty acids), fasting blood glucose and oral glucose tolerance tests, red

blood cell transketolase activity, basal metabolic rates, thyroidal I-131 uptake, urinary 17-ketosteroid and 17-ketogenic steroids, urinary nitrogen and creatinine, plasma and adipose fatty acid analysis by liquid gas chromatography, total body water (D_2O technique) and body composition. Data are incomplete at this time.

Summary and Conclusions:

Available data are being analyzed statistically, but a large amount of data is still outstanding.

List of Publications:

None at this stage of the investigation.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-20 A Study on Feeding of a Uniform Microcrystalline
Cellulose--its Digestibility and Effect on
Digestibility of Other Macronutrients

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic, Biochemistry and Bioenergetic
Divisions

1 July 1961 - 30 June 1962

John E. Canham, Lt. Col, MC
William T. Nunes, Maj, MC
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-20 A Study on Feeding of a Uniform Microcrystalline Cellulose--its Digestibility and Effect on Digestibility of Other Macronutrients

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: John E. Canham, William T. Nunes, Elizabeth M. Nevels, Richard S. Harding, and Ernest E. Preston

RCS MEDDH-288

Unclassified

The effect of adding a uniform microcrystalline cellulose at the levels of 10, 20, and 30% to a basal synthetic diet was evaluated to determine: the digestibility of a pure cellulose, influence on the digestibility of other nutrients, and any alteration in metabolism produced. Eight human subjects were utilized. Evaluation and analysis of data is not complete at the time of this report.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-20 A Study on Feeding of a Uniform Microcrystalline Cellulose--its Digestibility and Effect on Digestibility of Other Macronutrients

Description:

The increasing world population has caused investigators to explore the potential usability of various natural organic substances as possible foodstuffs. Cellulose, a polysaccharide component of the crude fiber of plants, is known to be unaffected by mammalian enzymatic digestion, but it is digested by certain cellulolytic microorganisms. Results of previous cellulose digestibility studies in man, dating back almost 100 years, have disclosed considerable variation with digestibility figures for cellulose, ranging from 0 to 79%. The amount of cellulose ingested in the above studies was generally quite small. Hence, cellulose has never been considered to contribute any significant number of calories to the human. Recently, a microcrystalline cellulose of uniform size, which lends itself well to nutritional studies, has become available. A study was designed to evaluate the digestibility of this pure microcrystalline cellulose in humans and its effect upon the absorption of the other macro- and micro-nutrients of the diet.

Progress:

During January and February, eight normal human subjects on the Metabolic ward were maintained on a basal synthetic diet calculated to yield 2,000 Calories/day. After two weeks on the basal diet, cellulose was added at successive intervals to equal 10, 20, and 30% by weight of the total diet. Urine and feces were collected to determine nitrogen balance and digestibility. Blood was taken periodically to determine any effect upon lipid, carbohydrate, and protein metabolism. Urinary excretion of vitamins B₁, B₂, B₆, and niacin were determined. The data are now being assembled and evaluated.

Summary and Conclusions:

The data from this study have not undergone sufficient analyses for definite conclusions. Though all subjects did lose weight on the 2,000 Calorie synthetic diet, there is a strong suggestion that in some subjects digestion of cellulose did occur to a significant degree. Nitrogen balance and fat digestibility were unaffected during the period of study. Upon completion of the data analyses, future studies will be planned if indicated.

List of Publications:

None at this stage of the investigation.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 3-21 Plasma Non-esterified Fatty Acid Response
to a Glucose Load in Normal, Diabetic, and
Obese Hyperglycemic Individuals

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Metabolic Division

1 July 1961 - 30 June 1962

William T. Nunes, Maj, MC
John E. Canham, Lt. Col, MC
Clyde A. Wilber, SSG

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-21 Plasma Non-esterified Fatty Acid Response to a Glucose Load in Normal, Diabetic, and Obese Hyperglycemic Individuals

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: W. T. Nunes, J. E. Canham, and C. A. Wilber

RCS MEDDH-288

Unclassified

An investigation of the plasma non-esterified fatty acid response to oral ingestion of a glucose load in various diabetic and non-diabetic individuals is being carried out. The test is carried out in subjects in the fasting state and all hypoglycemic agents are withheld prior to the administration of a measured dose of glucose. Plasma non-esterified fatty acid levels are determined in a fasting specimen and then 30 minutes, 1, 2, 3, and occasionally, 4 hours after the glucose is given.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 3-21 Plasma Non-esterified Fatty Acid Response to a Glucose Load in Normal, Diabetic, and Obese Hyperglycemic Individuals

Description:

The plasma non-esterified fatty acid levels of diabetics and non-diabetic individuals are measured following an oral load of glucose.

Progress:

A test is being carried out on selected diabetic and non-diabetic individuals in which, after an overnight fast, the subject is given an oral dose of 150 grams of glucose. Diabetics receiving hypoglycemic agents are instructed to withhold medication at least 12 hours prior to the test. Specimens include: a fasting plasma level of non-esterified fatty acids followed by a 30-minute, 1, 2, 3 and, occasionally, 4-hour determination. Fifteen determinations have been carried out. Results obtained thus far show the prompt, expected fall in the NEFA of normal subjects but variable responses in diabetics of varying severity.

Summary and Conclusions:

The fall in plasma NEFA in normal subjects is consistent and predictable following an oral dose of glucose. In diabetics of varying severity, there is a response which varies from normal

to no fall but rather a gradual rise in plasma non-esterified fatty acids.

List of Publications:

None at this stage of the investigation.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 4-6 Microbiological Support for Nutritional and
Miscellaneous Research

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Microbiology Division

1 July 1961 - 30 June 1962

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Lt Col W. C. Morse, MSC
O. L. Weiser, M. S.
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Sp4 C. A. Meyers

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 4-6 Microbiological Support for Nutritional and Miscellaneous Research

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Col L. R. Kuhn, MSC, Lt Col W. C. Morse, MSC, O. L. Weiser, M. S., J. S. Emerson, Sp4 C. A. Meyers

RCS MEDDH-288

Unclassified

Increased yields of C-14 tagged Chlorella are expected because of improvements in culture methods. A method for finding and counting cellulase-producing bacteria in stools was devised and is being used to determine changes in cellulolytic flora in Avicel-fed volunteers.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 - Metabolism and Nutrition

Sub-task No. 4-6 Microbiological Support for Nutritional and Miscellaneous Research

Description;

- a. Production of Chlorella containing radioactive carbon C^{14} .
- b. Identification and enumeration of cellulase-producing bacteria.

Progress;

a. A closed system for culturing Chlorella, one of the blue-green algae of interest in nutrition studies, has been developed which permits continuous addition of fresh medium and should permit continuous harvest of algae in the active phase of growth. This will yield larger amounts of Chlorella per unit volume of medium, and it will be applied to culturing Chlorella in medium containing "tagged" carbon (C^{14}) for the Chemistry Division to be used for nutritional studies.

b. 1. The Metabolic Division began a study of the nutritional utilization of Avicel, a microcrystalline cellulose. The Microbiology Division was asked to study methods for detecting and counting cellulose-digesting bacteria in the stools of volunteers fed this product. Although slow and laborious procedures were found, none were practical for the purpose of this study. Accordingly, media with Avicel and other simple cellulose materials were compared, using a culture of a known cellulose-digester. No practical method using Avicel itself could be devised, but recently a method employing a carboxy-methyl-cellulose in an agar overlay was found to be promising. With this

technique, countable depressions appear in the superimposed medium corresponding with colonies of bacteria digesting cellulose.

2. Six fecal specimens from an Avicel-fed volunteer have been studied with this technique. Three stools obtained and frozen before the Avicel regimen began, showed cellulase-producers ranging from 1,000 per gram to 200,000 per gram. Three stools obtained after Avicel was eaten contained 2.5, 5, and 7 million cellulose-digesters per gram. Stools from the other volunteers are now being studied as well as the organisms isolated. Better methods also are being sought.

Summary and Conclusions:

a. Increased yields of C-14 tagged Chlorella are anticipated following improvements in culture methods.

b. A method for finding and counting cellulase-producing bacteria in stools was devised and is being used to determine changes in cellulytic flora in Avicel-fed volunteers.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02

Metabolism and Nutrition

Sub-task No. 5-6 Measurement of Total Water Intake
in Field Using Tracer Method

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

Thomas H. Allen, Ph.D.
and
Harry J. Krazywiski, M.S.
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Robert W. Allard, SP4

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 5-6 Measurement of Total Water Intake in Field
Using Tracer Method

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Thomas H. Allen, Harry J. Krazywicki, Kenneth S.K. Chinn
and Robert W. Allard

RCS MEDDH-288

Unclassified

Through collaboration with other investigators,
current studies of total water intake in soldiers
using a deuterium washout method will shortly include
results at an additional geographic location. Deuterium
oxide to water ratios on samples from soldiers who were
being acclimatized in Greenland are still being analyzed.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 5-6 Measurement of Total Water Intake in Field
Using Tracer Method

Description:

Utilizing a deuterium tracer method the total water intake in military personnel engaged in various activities at different geographical locations is noted. From the various rates of water intake the range between the maximum and minimum water use may be determined.

Progress:

Through cooperation with other investigators, further information is being obtained on total water intake in Greenland.

Capt. Robert J. T. Joy, MC, of the USAMRNL, Ft. Knox, Kentucky, gave heavy water and obtained urine samples in a group of U. S. Army soldiers being tested by him while living in a simple shelter and traveling on the ice cap in Greenland. The ratio of $\text{HDO}:\text{H}_2\text{O}$ is being measured, and from this the total water intake will be calculated. The analyses, which are time-consuming, are still in progress.

The results on men at this location will be compared with data already obtained on soldiers at several other locations. Temperature, humidity, body water, pool size and level of physical exertion will be compared.

5-6

Summary and Conclusions:

No conclusions at present. Further opportunities for such studies are awaited, to permit the accumulation of sufficient data.

List of Publications:

None at this state of the investigation.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-1 Histopathology of Laboratory Animals

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Pathology Division

1 July 1961 - 30 June 1962

Lt. Col. Samuel W. Thompson, II, V.C.
Capt. Ronald D. Hunt, V.C.
Capt. John F. Ferrell, V.C.
Lt. Dean C. Frey, V.C.

Consultant:

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ABSTRACT

Project No. 6X60-01-001

INTERNAL MEDICINE

Tast No. 02 Metabolism and Nutrition

Sub-task No. 6-1 Histopathology of Laboratory Animals

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Samuel W. Thompson, II, John F. Ferrell, Ronald D. Hunt
Dean C. Frey and Charles L. Davis

RCS -MEDDH-288

Unclassified

The following studies were conducted under Sub-task No.

6-1 during the period covered by this report as follows:

- (1) Histopathology of Rats Fed Irradiated Foods. A paper, describing the spontaneous tumors found in aged rats was published.
- (2) Histopathology of Nodular Lymphoid Hyperplasia in the Lungs of Guinea Pigs. Histopathologic examination of available tissues was completed and a paper was published.
- (3) Spontaneous Regional Ileitis in Rats. Histopathologic studies were made of tissues from all available affected animals and a manuscript was published.
- (4) Spontaneous Endometrial Hyperplasia in the Guinea Pig. This hitherto unreported condition has been recognized and a histo-

pathologic study made. A paper was published.

(5) Incidental Lesions of Academic Interest Observed in

Surgical Animal Subject: A number of interesting incidental conditions, some of which were hitherto unreported, have been recognized in animals used for surgical research. Because of their academic interest, these are studied and reported in the appropriate literature.

BODY OF REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-1 Histopathology of Laboratory Animals

Sub-task No. 6-1 is a general purpose sub-task under which the various studies conducted on the histopathology of laboratory animals are grouped. The studies are conducted as dictated by the need of the pathologist to gain an insight into the diseases of laboratory animals so as to be able to evaluate the lesions which occur in test animals employed in the various tasks assigned to the divisions of this laboratory. The task is under general protocol, however, formal protocols are not developed for individual studies. The studies conducted during the period covered by this report are as follows:

1. Histopathology of Rats Fed Irradiated Foods. A paper describing 62 neoplasms, found in 52 of 125 rats which lived a normal lifespan, on a 100% composite irradiated diet, was published. Ten of the neoplasms had not previously been described as occurring in the rat.

The thyroid glands of the rats in the above study were serially sectioned, mounted on 35 mm. film stock, and studied microscopically. A special report on the evaluation of light cell adenomas in the thyroid glands was prepared.

2. Histopathology of Nodular Lymphoid Hyperplasia in the Lungs of Guinea Pigs. Histopathologic study of all guinea pig lung tissue collected in prior years was completed. Several specimens of lung tissue from guinea pigs born and raised in a germ free environment were examined and these also were found to contain the lesion.

It has been determined that the lung lesions observed are primarily lesions of pulmonary blood vessels which may have been inflammatory in nature. A paper, describing the gross and histologic appearance of the lesion, was published.

3. Spontaneous Regional Ileitis in Rats. A paper reporting this apparently new disease has been published. Recent shipments of rats have not been affected with this disease and this study is idle at the present time. If, at some later date, affected rats can be obtained a search for the etiologic agent will be made using animal inoculation and tissue culture technics.

4. Spontaneous Endometrial Hyperplasia in the Guinea Pig. A paper has been published describing the spontaneous occurrence of endometrial hyperplasia in 5 of 15 mature virgin females used in a tuberculosis prophylaxis study. Out of the many hundreds of guinea pigs necropsied in this laboratory, the condition has only been seen on this occasion. It is similar in gross and histologic appearance to the endometrial

hyperplasia in humans, dogs and cats. Because of the presence of ovarian follicular cysts in the affected animals, a hyper-estrogenic etiology is postulated.

5. Incidental Lesions of Academic Interest Observed in Surgical Animal Subjects. From time to time incidental lesions which are deemed worthy of publication, are found in dogs, cats and other species used for surgical research.

a) A tumor was found at the bifurcation of the carotid artery of a surgical research dog. Angiograms were made and the study was completed by the histopathological diagnosis of ganglioneuroma. Because of the completeness of the clinical, radiographic and pathologic work up and because of the rarity of the neoplasm, a report is being prepared for journal publication.

b) During the year, a paper was published on a liposarcoma in a dog.

c) A case of secondary hypertrophic osteoarthropathy was found in a dog referred for necropsy. Because of the completeness of clinical chemistry, radiographs, angiograms, bronchograms and pathologic work up a paper is being prepared for journal publication.

List of Publications:

1. Geil, R. G.; Davis, C. L. and Thompson, S. W.: Spontaneous Ileitis in Rats - A Report of 64 Cases, Am. J. Vet. Res., 22

(Sep 61), p. 932.

2. Thompson, S. W., Huseby, R. A., Fox, M. A., Davis, C. L. and Hunt, R. D.: Spontaneous Tumors in the Sprague-Dawley Rat., J. Nat. Cancer Inst., 27, #5 (Nov. 61), p. 1037.
3. Geil, R. G. and Davis, C. L.: Spontaneous Endometrial Hyperplasia in Guinea Pigs - A Report of 6 Cases, Am. J. Vet. Res., 23 (Mar 62), p. 362.
4. Thompson, S. W., Hunt, R. D., Fox, M. A., and Davis, C. L.: Perivascular Nodules of Lymphoid Cells in the Lungs of Normal Guinea Pigs, Am. J. Path., 40 (1962), p. 507.
5. Garvin, C. H. and Frey, D. C.: Liposarcoma in a Dog, J.A.V. M.A., 140, #10 (15 May 62), p. 1073.

List of Papers Presented:

1. Thompson, S. W., Ferrell, J. F. and Frey, D. C.: Clinical Seminar on Small Animal Pathology presented to the Denver Area Veterinary Medical Association, October, 1961.

Special Reports:

Special Report on the Evaluation of Light Cell Adenomas in the Thyroid of Rats Fed Irradiated and Non-irradiated Diets investigated by Lt. Col. S. W. Thompson, V.C. and Capt. Ronald D. Hunt, V.C., report prepared by, S. W. Thompson, R. D. Hunt, J. F. Ferrell, and D. C. Frey.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-2 Development and Application of Histochemical
Methods to Biological Research

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Pathology Division

1 July 1961 - 30 June 1962

Lt. Col. Samuel W. Thompson, II, V.C.
Capt Ronald D. Hunt, V.C.
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ABSTRACT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-2 Development and Application of Histochemical
Methods to Biological Research

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961- 30 June 1962

Authors: Samuel W. Thompson, II, Ronald D. Hunt, John F. Ferrell
and Charles L. Davis

RCS -MEDDH-288

Unclassified

The following studies were conducted under Sub-task No. 6-2
during the period covered by this report, as follows:

(1) Histochemistry of I.V. Fat Pigment - a variety of enzyme
histochemical methods have been adapted to tissues from animals
on the various intravenous alimentation studies.

(2) Autoradiography - this technic is being applied to a
study of thyroid function in the rat.

(3) Microscopic Histochemical Studies of Canine Distemper
and Infectious Canine Hepatitis Inclusions in Dogs. A study is
under way to compare histochemically the viral inclusions of
Canine Distemper and Infectious Canine Hepatitis.

(4) Electron Microscopy Studies of I.V. Fat Pigment. With the aid of Dr. Hans Popper, Mt Sinai Hospital, New York, I.V. fat pigment in the tissues of rats and rabbits is being studied with the aid of electron microscopy. A report has been published.

(5) Histochemical Study of Sheep Rumenal Pigment. Abnormal pigment of the rumen epithelium and submucosa associated with particular experimental rations was studied by histochemical means to aid in its characterization.

(6) Consultation Service. Aid in the application of histochemical technics in the diagnosis of certain types of lesions was provided to other governmental agencies and research institutions as well as hospitals.

BODY OF REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-2 Development and Application of Histochemical
Methods to Biological Research

Sub-task No. 6-2 is a general purpose sub-task under which the various studies conducted in the development and application of histochemical methods are grouped. These studies are conducted as required by the pathologists to develop new methods or modifications of existing histochemical and histopathological methods to meet the needs of various tasks assigned to the divisions of this laboratory and SGO investigators under contract. The histochemical studies are conducted to gain additional insight into the diseases of laboratory animals and thus provide the pathologists with a broader base of knowledge with which to interpret their observations in their service to other investigators of this laboratory. The studies conducted during the period covered by this report are as follows:

1. Histochemistry of I.V. Fat Pigment: The histochemical methods previously used to characterize I.V. fat pigment are being performed on tissues from rats and rabbits following the infusion of several newer fat emulsions (Southern Regional and Baxter Laboratories). The purpose of these studies is to

determine whether the pigment associated with these emulsions is identical to that produced by Lipomul and other previously tested emulsions. At the present time all data indicate that all I.V. fat pigments have the same histochemical properties.

Methods for the specific demonstration of acid phosphatase, alkaline phosphatase, beta glucuronidase, and adenosine tri-phosphatase have been performed on tissue sections of rat and rabbit liver from animals receiving fat emulsions to determine any change in amount and distribution of these enzymes in hepatocytes. It has been shown that hepatic and Kupffer cells which contain I.V. fat pigment have greater than normal amounts of acid phosphatase and adenosine tri-phosphatase activity.

2. Autoradiography. Investigations of thyroid function in the rat are being conducted with the aid of autoradiography. An attempt is being made to determine whether all follicles of a thyroid gland respond similarly to various means of suppressing and stimulating the gland as a whole. The follicular response is being measured by the amount of radioiodine incorporated into individual follicles. A paper is in preparation.

3. Microscopic Histochemical Studies of Canine Distemper and Infectious Canine Hepatitis. A large series of cases of Canine Distemper and Infectious Canine Hepatitis from this laboratory have been reviewed and suitable tissues selected for histochemical study. Protein, carbohydrate, and lipid

histochemical technics are being performed. The histochemical characteristics of nuclear and cytoplasmic inclusions produced by the same virus (Canine Distemper) in various tissues are being compared. A comparison is also being made between the inclusion bodies of the two diseases. The results of this study will be reported in an appropriate journal.

4. Electron Microscopy Studies of I.V. Fat Pigment.

This study is being carried out in collaboration with Dr. Hans Popper, Dr. Fenton Schaffner, Dr. Tibor Barka and Dr. Walter Neglia of Mt Sinai Hospital, New York. Representative live rats taken from groups which had received 20 daily infusions (15 ml/kg body wt.) of Lipomul were shipped to Dr. Popper in September 1960.

It has been determined that the pigment exists in a globular form within a lysosomal-like structure in the cytoplasm of hepatocytes and Kupffer cells. It has also been shown that there is an increase in the number of normal lysosomes in the cells containing I.V. fat pigment. These results have been reported.

Specimens of liver from rabbits on current alimentation studies are being sent to Dr. Popper for further electron microscopic studies.

5. Histochemical Study of Sheep Rumenal Pigment.

Abnormal pigments have been observed within the rumenal

epithelium and submucosa of sheep on various types of experimental (high-protein) diets at Iowa State University. Selected tissue specimens were obtained for histochemical studies.

Various protein, carbohydrate, and lipid histochemical technics have been applied to this pigment in an attempt to characterize it. Characterization of the pigment is of interest due to the diets associated with its occurrence and the possible insight that may be gained into nutritional pathology of laboratory animals.

A report is being prepared with Dr. Frank Ramsey, and Dr. Walter Woods of Iowa State University, for Journal publication.

6. Consultation Service. Specimens are received from other governmental, academic and research institutions as well as hospitals for assistance in histochemical problems. Such services are rendered to:

- a) Fitzsimons General Hospital: Preventive Medicine - rabies diagnosis
- b) Iowa State University: Aid in the characterization of pigment observed in the rumens of sheep.
- c) Medical School, University of Louisiana: Aid in identifying I.V. fat pigment.
- d) Medical School, Vanderbilt University: Aid in identifying I.V. fat pigment.

List of Publications:

1. Thompson, II, S. W.; Bodgon, T. R. and Yost, D. H.: Some Histochemical Studies of "Cloisonné Kidney" in the Male Angora Goat, Am. J. Vet. Res., Vol. 22, No. 89, July 1961. p. 757.
2. Geil, R. G.: A simple technic for the salvage of tissue sections from broken microslides, Tech. Bull. Reg. of Med. Technol. 31, 1961, pp 195-196.
3. Thompson II, S. W.: "Selected Histochemical and Histo-pathological Methods," to be published by Charles C. Thomas, Springfield, Illinois in 1962. Increased to 14 chapters totaling 1000 pp. (approx.) and 250 il., 11 of 14 chapters completed.
4. Schaffner, F., Neglia, W. and Thompson II, S. W.: Electron Microscopy and Histochemistry of I.V. Fat Pigment, Federation Proceedings, April 1962.

List of Papers Presented:

1. Thompson II, S. W.: Newer Applications of Histochemistry, to Am. Col. Vet. Path, AVMA Convention, Detroit, Mich., Aug. 1961.
2. Thompson II, S. W.: Lectures in histochemistry to pathology residents, Fitzsimons General Hospital, biweekly.
3. Thompson II, S. W.: Applications of Histochemistry to Pulmonary Pathology, TB Symposium. Fitzsimons General Hospital, Sep. 1961.

4. Thompson II, S. W.: Histochemistry of I.V. Fat Pigment,
Am. Assoc. for Study of Liver Diseases, Chicago, Nov,
1961.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-3 Assessment of Toxicity of Various Preparations
for Intravenous Alimentation. Study B. Toxicity
of Intravenous Fat Emulsions

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Pathology Division

1 July 1961 - 30 June 1962

Lt. Col. Samuel W. Thompson II, V.C.
Major Mayna R. Allen, A.N.C.
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ABSTRACT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-3 Assessment of Toxicity of Various Preparations
for Intravenous Alimentation. Study B. Toxicity
of Intravenous Fat Emulsions

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Samuel W. Thompson II, Mayna R. Allen, John F. Ferrell,
Ronald D. Hunt and Charles L. Davis

RCS - MEDDH-288

Unclassified

The following study was conducted under Task No. 02, Sub-task
No. 6-3, during the period covered by this report:

Toxicity of Intravenous Fat Emulsions. Studies are being
conducted in animals to assess the acute and chronic toxicity of
several intravenous fat emulsions with special emphasis on hepa-
tic function and micro-pathology. These studies are being con-
ducted in association with Mt Sinai Hospital, New York, New York,
Vanderbilt University, Nashville, Tennessee and Louisiana State
University, New Orleans, Louisiana.

BODY OF REPORT

Project No. 6X60-01-001

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-3 Assessment of Toxicity of Various Preparations for Intravenous Alimentation. Study B. Toxicity of Intravenous Fat Emulsions

Description:

Subtask No. 6-3 is a general purpose sub-task under which all biological toxicity testing, and related histopathology, of products intended for intravenous administration are grouped. This is essentially a service function. The products to be tested are furnished by other investigators of this laboratory or SGO. The task is outlined in a broad scope protocol and special detailed protocols are prepared for each product submitted for testing. These latter protocols are approved by the requestor as well as other service facilities of this laboratory prior to the initiation of a given study. During the period covered by this report a study was conducted on intravenous fat emulsions.

Progress:

Toxicity of Intravenous Fat Emulsions. A joint study involving Mt. Sinai Hospital, New York, Louisiana State University, Vanderbilt University and USAMR&NL is being conducted to determine the acute and chronic effects of various intravenous fat emulsions, especially as to their

production of pigments within the liver and resultant hepatic disease. Four emulsions and their emulsifying systems are being tested: SR 695, SR 695-prepared by Baxter Laboratories, "Intralipid," and a synthetic triglyceride.

The emulsions are being administered to rabbits at the rate of 15 ml/kg body weight, using a 15% emulsion. Infusion time is approximately 105 minutes. Each rabbit will receive 30 infusions. Throughout the period of each phase of the study, extensive clinical and laboratory data is collected. Several liver function tests are used on this study and include: radioactive gold, radioactive (I^{131} tagged) Rose Bengal, and BSP (depending on study and group). Liver biopsies are also done on all test animals and specimens are submitted to Mt Sinai Hospital (Drs. Hans Popper, Fenton Schaffner, and Tibor Barka) for electron microscopic studies. Specimens are also retained by the Pathology Division, USAMRENL for enzyme histochemical studies. Periodic liver function tests and liver biopsies will be performed for periods up to six months following the completion of infusions. Time of sacrifice of animals will depend on findings of these tests.

Louisiana State University (Drs. Isidore Cohn and Mohammed Atik) is infusing the same materials into dogs and is sending biopsy and necropsy material to the Pathology Division, USAMRENL for histochemical and histopathological studies.

Vanderbilt University (Dr. Raymond Meng) is infusing "Intralipid" into dogs and rats, at the rate of 15 ml./kg body weight. Each animal will receive 30 infusions. Biopsy and necropsy material from the dogs, and live rats, are being sent to the Pathology Division, USAMR&NL for histochemical and histopathological studies and for transshipment to Mt Sinai Hospital if electron microscopy is deemed appropriate.

Conclusions:

A joint evaluation of all data will be performed at the completion of these studies, and a joint report prepared. This will be accomplished at a meeting of all participants at USAMR&NL in the second quarter of FY 1962.

List of Publications:

Thompson II, S. W.: Long Term Effects of I.V. Fat, Gastroenterology, Apr. 1962.

List of Papers Presented:

1. Thompson II, S. W.: Long Term Effects of I.V. Fat, to Surgeon General's Task Force, Sep. 1961 and Am. Assoc. for the Study of Liver Disease, Nov. 1961, and Am. Col. of Vet. Tox., Salt Lake City, Jan. 1962.
2. Hunt, R. D.: Effects of I.V. Fat Pigment in the Livers of Animals, to Society for Experimental Biology and Medicine Rocky Mtn. Region, Apr. 1962.
3. Hunt, R. D.: Studies on I.V. Fat Pigment to MEND Symposium, USAMR&NL, May 1962.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-6 Carcinogenicity of Foods Preserved by Irradiation

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Pathology Division

1 July 1961 - 30 June 1962

Lt. Col. Samuel W. Thompson, II, V.C.
Capt. Ronald D. Hunt, V.C.
Capt. John F. Ferrell, V.C.
Lt. Dean C. Frey, V.C.

Consultant:

Denver, Colorado
Charles L. Davis, D.V.M., Vet. Dipl.

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ABSTRACT

Project No. 6X60-01-001

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-6 Carcinogenicity of Foods Preserved by Irradiation

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado.

Authors: Samuel W. Thompson, II, Ronald D. Hunt, John F. Ferrell,
and Dean C. Frey.

RCS - MEDDH-288

Unclassified

This study is designed to further evaluate the reproducibility, pathogenesis, and etiology of the left auricular lesions originally observed by Dr. Harry Monsen (DOA Med. Res. Contract #CA 49 007 MD 794) in Cb and Strong A strains of mice fed a composite 100% irradiated diet. At present all animals have been sacrificed on two phases of the study and 485 remain to be sacrificed. Statistical analysis of results awaits the completion of histopathological reports on all animals on the study. At the present time no conclusions can be drawn regarding the incidence of lesions in experimental versus control animals.

BODY OF REPORT

Project No. 6X60-01-001

Task No. 02 Metabolism and Nutrition

Sub-task No. 6-6 Carcinogenicity of Foods Preserved by Irradiation

Description:

This project is a repeat (at the request of the SGO) of the study conducted by Dr. Harry Monsen of the Department of Anatomy, University of Illinois, College of Medicine, on Department of the Army Medical Research Contract #DA 49 007 MD 794. He reported the occurrence of a lesion which afflicted the left auricle of the heart of some mice of the Cb and Strong A strains. The incidence rate of this lesion incriminated the irradiated foods which were fed. The study being conducted at this laboratory is designed to further evaluate the reproducibility, pathogenesis and etiology of the left auricular lesions originally observed by Dr. Monsen. It is divided into 4 phases: (1) breeding of the required strains of mice; (2) repeat of Dr. Monsen's original protocol; (3) pathogenesis and (4) etiology.

The irradiated and the non-irradiated control diet consists of a composite of pork, chicken, milk (evaporated), white potatoes, and carrots (blanched), to which is added commercial vitamin and salt mixtures. A commercial laboratory rodent pellet diet is also being fed as a second control.

Progress:

Phase I - The breeding of the required number of mice has been completed and this phase of the project has been discontinued. All breeding stock mice whose offspring were placed in another phase of the experiment are being allowed to live out their lifespan. These mice will receive a complete necropsy and histopathological examination at the time of their death. One hundred twelve mice remain to be sacrificed.

Phase II - Due to a change in the age of occurrence of heart lesions reported by Dr. Monsen, the following change in protocol has been made and approved: the sacrifice dates for this phase have been changed from a single 200 day sacrifice to 200, 300, 400, 500, and 600 day sacrifices. Twelve hundred Cb and 1200 Strong A mice were used in this phase and were divided into three equal groups of paired litter mates according to diet: irradiated, non-irradiated and pellet control. All animals on this phase have now been sacrificed.

Phase III - All mice of the Cb strain which were originally scheduled for this phase have been put on test. For the reason cited above, (Phase II), this phase was extended from 200 days to 300 days in length with mice being sacrificed at 25 day intervals. At 0 days and every 25 days thereafter up to and including 100 days, 6 females and 6 males were sacrificed. Beginning at 125 days and every 25 days thereafter

up to and including 300 days, 3 females and 3 males were sacrificed. All 108 mice scheduled for this phase have been sacrificed.

Phase IV - Due to the change in the age of occurrence of heart lesions in Dr. Monsen's report the following changes in protocol were made and approved: the number of mice on each diet component was increased from 250 per group to 400 per group, increasing the total number of mice from 1000 to 1600; the single 100 day sacrifice originally proposed was changed to sacrifices of equal numbers of mice at 100, 200, 400 and 600 days. The mice are being fed a total of 20 diets. One ingredient of each diet being irradiated. Each of these 5 diets is then divided into four groups: cooked with vitamins, cooked without vitamins, uncooked with vitamins and uncooked without vitamins. Three hundred four animals remain to be sacrificed, with the last scheduled sacrifice date 6 Oct, 1962.

At the request of Colonel Hursh, a total of 84 mice of the Cb strain were placed on an irradiated diet in October 1960 similar to Phase II with the exception that the milk component was stored at -10°F rather than at room temperature. To date 16 of these mice have died, leaving 68 mice still on the study.

At the suggestion of Dr. Monsen 72 mice of the Cb strain were placed on a milk diet fortified with vitamin mix on 19 August 1960. This milk was furnished by Dr. Monsen from his stock. The animals

on this study are divided as follows:

Group 1 - 18 mice - uncooked irradiated milk,

Group 2 - 18 mice - cooked irradiated milk,

Group 3 - 18 mice - uncooked non-irradiated milk,

Group 4 - 18 mice - cooked non-irradiated milk.

To date 71 mice have died leaving 1 on the study.

Dr. Monsen ran a companion study.

At the suggestion of Major Berg, QMF&CI a total of 72 mice of the Cb strain were fed a diet similar to the Phase II diet. However, these mice were fed in bean pots rather than disposable containers (to rule out or rule in effects of sanitary aspects). Thirty-six were fed the irradiated diet and 36 were fed the control diet. All mice have been sacrificed.

Summary and Conclusions:

A total of 4376 mice have been placed on the experiment and a total of 3891 mice have died or been sacrificed to date.

Statistical analysis of results awaits the completion of histopathological reports on all animals on the study. At the present time no conclusions can be drawn regarding the incidence of lesions in experimental versus control animals. Grossly recognizable dilatation and/or rupture of the left auricle and atrium, as described by Dr. Monsen, has not been seen. Thrombosis has been observed in microscopic sections of the heart in several animals. Calcification, usually of the right ventricle, and

focal chronic myocarditis are seen rather frequently. Other lesions which have been observed routinely are: urolithiasis of the kidney, adrenal cortical hyperplasia, reticuloendothelial hyperplasia of various lymph nodes, and lung tumors. A high incidence of mammary carcinomas and fibrosarcomas is noted in the animals from the breeding colony.

List of Publications:

None.

List of Papers Presented:

Thompson, S. W. II, Irradiated Food and Cardiac Lesions in Mice, MEND Conference, USAMRNL, May 1962.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 04 - Pharmacology of the Combat Soldier

Sub-task No. 1-1 Physiological Measurements of
Metabolic Function in Man

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio

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Cincinnati, Ohio

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-1 Physiological Measurements of Metabolic Function in Man

Collaborative Study with the Department of Physiology, University of Illinois, Urbana Illinois, and the Veterans' Administration Hospital, Cincinnati, Ohio.

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: C. F. Consolazio, R. E. Johnson and L. J. Pecora.

RCS MEDDE-288

Unclassified

A manuscript entitled "Physiological Measurements of Metabolic Function in Man" has been completed, submitted and accepted for publication by McGraw-Hill Publishing Company, Elakiston Division, New York City. This book deals primarily with the various physiological procedures used to measure metabolic function in man. Some of the areas covered in 14 chapters include respiratory metabolism, body composition, pulmonary function, metabolic and heat balances, and physical fitness and performance.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-1 Physiological Measurements of Metabolic Function in Man

Description:

This manuscript which contains 14 sections, deals primarily with the various physiological measurements used to measure metabolic functions in man.

Progress:

A final draft of this manuscript has been completed and accepted for publication with a September 1962 issue date by the Elakiston Division of the McGraw-Hill Publishing Company.

Summary and Conclusions:

The manuscript of a book entitled "Physiological Measurements of Metabolic Function in Man", under revision for several years, has been completed and is in the hands of the publishers (Elakiston Division of McGraw-Hill Publishing Co.). The sections of this book are as follows: (1) Respiratory Metabolism, (2) Analysis of the Respiratory Gases, (3) The Physical Chemistry of the Blood Gases, (4) The Analysis of Blood Gases and pH, (5) Measurement of Pulmonary Function, (6) Body Composition, (7) Measurement of Body Composition, (8) Computation of Metabolic Balances, (9) Physical Fitness and Performance, (10) Methods for Testing Physical Fitness, (11) Meteorological Measurements, (12) Heat Balance, (13) Limits of Variability of Physiological Measurements, and (14) Miscellaneous Tables. This should be of value to researchers studying the "pharmacology of the combat soldier".

Publication:

In press for 1962 publication.

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ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 04 - Pharmacology of the Combat Soldier

Sub-task No. 1-1f Development and Application
Of A Continuous Direct Reading Instrument for
Measuring Oxygen Consumption During Exercise

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

Richard A. Nelson
and
Bert M. Tolbert, Ph. D.
C. Frank Consolazio
LeRoy O. Matoush

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology and the Combat Soldier

Sub-task No. 1-1f Development and Application of A Continuous Direct Reading Instrument for Measuring Oxygen Consumption During Exercise

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Richard A. Nelson, Bert M. Tolbert, C. Frank Consolazio and LeRoy O. Matoush

RCS MEDDH-288

Unclassified

The apparatus which was previously developed for the continuous measurement of oxygen consumption and body temperature has been refined for ease in operation. A dry gas meter has been fitted with an electrical output and installed in the unit to replace the Pneumotachograph which was previously used. Two automatic timers have been installed, one which controls the movement of expired gases through the apparatus and one which controls the collection of the data on strip chart recorders. An additional gas inlet has been included in the apparatus to permit the gross analysis of respiratory gases which have been collected in respiration bags from subjects not connected to the analyzer.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-1f Development and Application of A Continuous Direct Reading Instrument for Measuring Oxygen Consumption During Exercise

Description:

The objective of this project has been to develop an apparatus for the continuous monitoring of expiratory gas volume, expiratory oxygen and carbon dioxide concentration, pulse rate and temperature on humans during rest and exercise.

Progress:

The oxygen consumption analyzer was used for measuring energy expenditure continuously for a period of three months. It was found that for long term measurements, that is longer than 15 minutes, the inherent instability of a pneumotachograph type flow meter necessitates the continuous recalibration of the instrument during use. This is undesirable since it prevents personnel from doing the other tasks necessary when measuring energy expenditure. A standard dry gas meter was fitted with a continuous type potentiometer on its dial. An electrical signal is tapped from this potentiometer and recorded on a strip chart recorder. In this manner, an expiratory gas flow is measured without the use of a complex electronic instrument thus eliminating the problem of instability. This unit was used with 12 subjects for two months without difficulty and with extremely good dependability.

A timing unit is being added which will accomplish the flushing of the instrument and the recording of the results with reproducible time elements. This unit consists of an interval timer which controls the length of time gas is pumped through the analyzer. A delay timer prevents the recorders from operating before the analyzer has been properly flushed of gas remaining from the previous sample, thus eliminating erroneous results.

Solenoid operated valves have been added which permit the selection of gas either from the subject connected to the analyzer or from a respiration bag containing previously collected gas. Regardless of which source of gas is used, it is dried and pumped through the analyzers in the same manner, thus preventing additional handling of the gas. This makes it possible to measure gross energy expenditure on subjects with collection bags while measuring a single subject continuously with the analyzer unit.

Summary and Conclusions:

The continuous oxygen consumption analyzer has been refined to allow long term sampling with less handling. A dry gas meter has been incorporated for the measurement of expiratory gas volumes and timers have been added to control the length of the samples. A modification permits the analysis of expired air, which has been collected in sampling bags, for oxygen and carbon dioxide concentration. When a suitable unit is found for the recording of pulse rates during exercise, this will be added to the unit.

Publications:

A laboratory report showing the wiring diagrams and other essential parts of the instrument will be prepared in the near future.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 04 - Pharmacology of the Combat Soldier

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Sub-task No. 1-9 Physiological and Biochemical
Evaluation Of Potential Anti-Fatigue Drugs:
(K and Mg Aspartic Acid Salts (Spartase) in Humans)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

C. Frank Consolazio
Richard A. Nelson
LeRoy O. Matoush
Gerhard J. Isaac

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ABSTRACT

Project No. 6X60-01-001

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-9 Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs:

K and Mg Aspartic Acid Salts (Spartase) in Humans

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: C. Frank Consolazio, Richard A. Nelson, LeRoy O. Matoush and Gerhard J. Isaac

RCS MEDDH-288

Unclassified

During the past few years several French investigators have reported the beneficial effects of aspartic acid salts as a means of reducing fatigue at the neuromuscular level. Laborit, et al. postulated that potassium and magnesium salts of aspartic acid, functioning in an intermediate position in the Krebs cycle, should provide an increase of metabolic efficiency or delay of metabolic exhaustion, and in support of this theory claimed increased endurance of swimming rats given K or mg-aspartate. As reported in last year's annual progress report, our Laboratory found no difference in swimming times (to exhaustion) between rats, or dogs, given aspartate salts, and control animals.

Investigation was extended to human subjects, using a commercial preparation of K and Mg salts of aspartic acid recently made available to physicians in this country, and described as an anti-fatigue agent. Twelve test subjects, who ranged in age from 20 to 48 years, performed a daily brisk treadmill walk for 30 minutes for a total of 9 weeks. A treadmill run was also performed during the 6th and 7th weeks and at the end of the control recovery period. In addition comparisons were made of vital capacity, maximum breathing capacity, breath holding, grip strength and Rheotome responses. The men were divided randomly into two groups and given aspartate or placebo in "double-blind" technique. The first two weeks were control periods to rule out the effect of training, the next 5 weeks were "therapy" of either the aspartic acid salts or the placebos, and the last two weeks a control recovery period.

Comparisons are now being made of the treadmill and other tests performed during the study.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-Task No. 1-9 Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs:

K and Mg Aspartic Acid Salts (Spartase) in Humans

Description:

This study was designed to investigate the effects of aspartic acid salts (another of the so-called anti-fatigue agents?), as a possible means of increasing endurance and prolonging the onset of fatigue.

Progress:

No new studies have been done with animals.

A study has been completed on 12 men for a period of 9 weeks. The men exercised 30 minutes a day, 5 days a week by walking briskly at 4.0 mph on a 3.5% grade. The study included a 2 week control period, a 5 week therapy period (6 men ingesting 2 gm of Spartase daily) and a 2 week recovery period. Energy expenditure was measured 2 days a week on each man, during the last 10 minutes of exercise and for 10 minutes of recovery following exercise. A maximum run was performed on the treadmill (at 6.8 mph at an 8.0% grade) at the end of the 6th, 7th and 9th weeks of the study.

Summary and Conclusions:

The effects of "Spartase" has been studied on men undergoing exercise on a motor-driven treadmill. Preliminary inspection of the data suggests that there is no difference between the "Spartase" and control groups, in any of the measurements made. It seems that under the conditions of this experiment "Spartase" had no effect on the ability of men to perform a fixed task.

Publication:

A manuscript is being prepared for publication.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 04 - Pharmacology of the Combat Soldier

Sub-Task No. 1-9a Physiological and Biochemical
Evaluation of Potential Anti-Fatigue Drugs: THAM

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

LeRoy O. Matoush
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SSG Juan B. Torres
Gerhard J. Isaac

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-9a Physiological and Biochemical Evaluation of Potential
Anti-Fatigue Drugs: THAM

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General
Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: LeRoy O. Matoush, Richard A. Nelson, C. Frank Consolazio,
Juan B. Torres and Gerhard J. Isaac

RCS MEDDH-288

Unclassified .

No new experiments testing the endurance of animals treated with the organic tris buffer, THAM, have been performed during the past year. In previously reported studies, infusion of THAM prior to the exercise gave no significant improvement in swimming times (to exhaustion) of dogs and rats. Plans and techniques have been developed to measure endurance times and physiologic parameters in dogs running on a treadmill when THAM is infused late in exhausting exercise as near the end point as can be predicted, or after exhausting exercise.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-9a Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs: THAM

Description:

Recent work with THAM (tris hydroxymethylaminomethane), an amine buffer, has shown that when administered intravenously during severe acidosis, it will combine with carbon dioxide very effectively and is able to maintain the blood pH within a narrow range near 7.40. Mahas has reported that the intravenous administration of THAM will increase the endurance time of dogs running on a treadmill at high speeds. It has been postulated that THAM will combine and neutralize the free lactic acid which accumulates in the blood stream during severe exercise and thus prevents the acidosis which would normally occur.

Progress:

This project remained inactive during the past year since further work was necessary (a) on the development of chemical procedures for measurement of THAM blood levels, (b) on the development of a suitable dog mask for measuring metabolic rates, and (c) on the training of the dogs on the treadmill.

In the first phase of this study the effect of THAM was evaluated to see whether it prolonged the swimming time of rats and dogs. In each of two studies 40 rats swam to exhaustion at 25 and 17°C and in the third study 4 dogs swam at 17°C.

Summary and Conclusions:

In study one (25°C), the controls had mean swimming times of 37.0 ± 25.2 minutes in comparison to the THAM-infused times of 32.4 ± 18.9 minutes. At 17°C, the control rats swam for 12.6 ± 2.4 while the THAM-infused rats averaged 13.6 ± 1.8 minutes. In the dog experiment the controls averaged 117 ± 70 while the THAM-infused averaged 123 ± 57 minutes. Under the conditions of this study, the swimming times of rats or dogs infused with THAM, did not differ significantly from the swimming times of the control animals.

The next phase of this study will be to infuse dogs with THAM after an exhausting performance on the treadmill and to observe their performance.

List of Publications:

The Effects of THAM on the Swimming Time of Rats and Dogs (In press)
LeRoy O. Matoush, Richard A. Nelson, C. Frank Consolazio, SSG Juan B. Torres
and Gerhard J. Isaac.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 04 - Pharmacology of the Combat Soldier

Sub-task No. 1-9c Physiological and Biochemical
Evaluation of Potential Anti-Fatigue Drugs:
(Wheat Germ Oil, Vitamin C and Vitamin E)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

LeRoy O. Matoush
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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-9c Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs

Wheat Germ Oil, Vitamin C and Vitamin E

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado.

1 July 1961 - 30 June 1962

Authors: LeRoy O. Matoush, C. Frank Consolazio, Juan B. Torres, and Richard A. Nelson.

RCS MEDDH-288

Unclassified

The primary object of this study was to evaluate the effects of the oral ingestion of wheat germ oil, vitamin E and vitamin C on the swimming times of rats (to exhaustion). As reported in last year's annual progress report, these rats were divided into 4 groups, including a control, and were allowed to swim at 25°C, carrying a 9 gm weight around the neck. There were no significant differences in the swimming times of the drug treated animals when compared to the control animals. No new experiments have been performed, but plans have been made, and the preparation "Octocoonol", a wheat germ oil extract, has been obtained for testing purposes.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-9c Physiological and Biochemical Evaluation of Potential Anti-Fatigue Drugs

Wheat Germ Oil, Vitamin C and Vitamin E

Description:

The objective of this study was to evaluate some of the "so called" anti-fatigue drugs, on rats swimming to exhaustion. They included wheat germ oil, vitamin E and vitamin C.

Progress:

No new experiments have been performed in this reporting period. Plans have been made to test another preparation "Octocosonol", a wheat germ oil extract. The producers claim that this substance is the compound that will prolong endurance and prevent the onset of fatigue. The detailed report in this area will be deferred pending completion of tests of "Octocosonol".

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 04 - Pharmacology of the Combat Soldier

Sub-task No. 1-13 The Response to Exercise As
Related to Age and Environmental Temperature

University of Indiana
Department of Physiology and Anatomy
Bloomington, Indiana

and

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Bioenergetics Division

1 July 1961 - 30 June 1962

David B. Dill, Ph. D.
C. Frank Consolazio

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ABSTRACT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 1-13 The Response to Exercise As Related to Age and Environmental Temperature

Collaborative Study with University of Indiana, Department of Physiology and Anatomy, Bloomington, Indiana, and U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado.

1 July 1961 - 30 June 1962

Authors: David B. Dill and C. Frank Consolazio

RCS MEDDE-288

Unclassified

The responses of two subjects to exercise were studied after a time lapse of 29 years. Environmental temperatures varied from 0 to 50°C and oxygen consumptions from the resting value to about 10 times BMR. In DHD, present age 70, the heart rates were about the same in easy and moderate work at temperatures up to 40°C, but much higher at 50°C. In FC, present age 50, at all work levels the heart rates were the same up to 20°C, lower at 30 and 40°C and higher at 50°C. The aerobic capacity of both subjects had declined about one-fourth. Thus the responses to the stress of extreme heat showed little loss while there was considerable loss in the ability to withstand the stress of hard work.

BODY OF REPORT

Project No. 6X60-01-001 INTERNAL MEDICINE

Task No. 04 Pharmacology and the Combat Soldier

Sub-task No. 1-13 The Response to Exercise as Related to Age and Environmental Temperature

Collaborative Study with University of Indiana, Department of Physiology and Anatomy, Bloomington, Indiana, and U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado.

Description:

The decline of the capacity for hard work with increasing age is well known. Whether there is necessarily a decreased ability to withstand other stresses such as heat is not certain. To investigate this question a study has been repeated on two subjects on the bicycle ergometer carried out 29 years ago. Subject FC is now 50 years old and DED 70.

Progress:

This study was completed at the University of Indiana and Army Medical Research Laboratory, Fort Knox, Kentucky during May 1961. Measurements included metabolic rate and pulse rate at 6 temperatures of 0° to 50°C and 6 levels of physical activity. The data has been analyzed and a group of curves constructed showing the heart rate for FC in rest and all grades of work up to an oxygen consumption of 2.6 liters/minute and at temperatures ranging from 0 to 50°C; and for DED up to an oxygen intake of 2.1 liters/minute. A report has been completed and is in press.

Summary and Conclusions:

It has been observed that in DED, age 70, the heart rates were approximately the same in the light to moderate work rates at environmental temperatures up to 40°C, but at 50°C the pulse rates were much greater. In FC, age 50, at all the work levels up to 20°C, the heart rates were the same, but at 30 and 40°C they were lower and at 50°C they were increased. It has been shown that the aerobic capacity of both subjects had declined about one-fourth. The responses to the stress of extreme heat showed little loss while there was a considerable loss in the ability to withstand the stress of hard work.

Publications:

1. The Response to Exercise as Related to Age and Environmental Temperature. D. B. Dill and C. F. Consolazio. American Physiological Society Annual Meeting, September 1961.

2. The response to Exercise as Related to Age and Environmental Temperature. D. B. Dill and C. F. Consolazio. J. Appl. Physiol. May - June 1962 (In press).

ANNUAL PROGRESS REPORT

Project No. 6X60-01-001

INTERNAL MEDICINE

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 6-3 Assessment of Toxicity of Various Preparations
for Intravenous Alimentation. Study A. Toxicity
of Tris Buffer.

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Pathology Division

1 July 1961 - 30 June 1962

Lt. Col. Samuel W. Thompson II, V.C.
Major Mayna R. Allen, A.N.C.
Capt. John F. Ferrell, V.C.
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ABSTRACT

Project No. 6X60-01-001

Task No. 04 Pharmacology of the Combat Soldier

Sub-task 6-3 Assessment of Toxicity of Various Preparations for Intravenous Alimentation. Study A. Toxicity of Tris Buffer

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Samuel W. Thompson II, Mayna R. Allen, John F. Ferrell, Ronald D. Hunt and Charles L. Davis

RCS - MEDDH-288

Unclassified

The following study was conducted under Task No. 04, Sub-task No. 6-3, during the period covered by this report:

Toxicity of Tris Buffer Administered Intravenously to Normal Laboratory Animals. Tris buffer has been administered to 8 rabbits and 1 dog for the purpose of determining histochemical and pathologic changes produced. In the rabbits the only lesions which could be attributed to THAM were local reactions to the infused material which resulted in ulceration of the ears.

The one dog which received THAM in dosage of 1.5 g/Kg daily, infused at a rate of 0.5 ml/Kg/min of 0.3 Molar solution, died 12 hours after the second infusion and showed acute toxic nephrosis on necropsy.

BODY OF REPORT

Project No. 6X60-01-001

Task No. 04 Pharmacology of the Combat Soldier

Sub-task No. 6-3 Assessment of Toxicity of Various Preparations for Intravenous Alimentation. Study A. Toxicity of Tris Buffer

Description:

Sub-task No. 6-3 is a general purpose sub-task under which all biological toxicity testing, and related histopathology, of products intended for intravenous administration are grouped.

Tris buffer (THAM) has been suggested as a potential anti-fatigue agent (or at least as a prototype of such a compound) because of its ability to buffer lactic and carbonic acids. Prior to possible use in humans, and concomitantly with animal physiological studies, more pharmacology and pathology studies in experimental animals were needed.

Progress:

Toxicity of Tris Buffer Administered Intravenously to Normal Laboratory Animals. Tris buffer was administered to 8 rabbits for a period of 20 days. Eight rabbits were used as controls and received normal saline to which 5 milli equivalents of KCl per liter was added. The test animals were given a 0.3 Molar solution of THAM at the rate of 0.5 gm/kg at 0.5 ml/min. for a total dose of 1.5 Gm. or 41 ml. for a 3.0 Kg animal and 2.0 Gm. or 55 ml. for a 4.0 Kg animal.

Four test and four control animals were sacrificed at the completion of the infusions. Equal numbers were held for 20 days before being sacrificed. Clinical, clinico-pathologic, necropsy, and histopathological findings were recorded. Special enzyme histochemical stains were done for the purpose of determining alkaline phosphatase, acid phosphatase, esterase, peptidase, DPN diaphorase, and TPN diaphorase activity in kidney tissue from experimental and control animals.

Of all the lesions observed in the test animals, a cellulitis and ulceration of the ears at the site of infusion was the only one that could be specifically attributed to the injection of THAM. Nephrosis was observed in one animal and may or may not have been due to the injection of THAM.

One dog received two infusions of a 0.3 Molar solution of THAM, each infusion totaling 1.5 gm/kg, at a rate of 4.36 ml/min. (90 min.) (a total of 392.5 ml. per infusion). Trembling and vomiting occurred during the first infusion. Following the infusion a generalized weakness was apparent. During the second infusion the same symptoms were observed, as was marked hematuria. Following the second infusion urine flow ceased and the dog's condition deteriorated rapidly. Apprehension was followed by weakness and unconsciousness with occasional stiffening of the extremities. The comatose animal died 12 hours postinfusion. The Histopathologic diagnosis was acute nephrosis. The BUN rose from 58.8 to 96.7 on the day of death and the creatinine rose from 2.87 to 5.93.

The dose and rate of administration of the Tris buffer was not greater than that previously reported to be tolerated in dogs, and the response of this animal was unusual but must be presumed to be associated with the infusions. Whether some unknown pre-existing disease, or a particular physiological state of the animal contributed to the reaction and death is not known, and this represents only one case, incompletely studied, but the experience at present further interdicts trials of the Tris buffer in normal humans in this laboratory as a possible anti-fatigue agent, even though total dosage in humans would be considerably lower. Its therapeutic use in appropriate patients, however, is not gainsaid.

Papers summarizing these and previous studies on the histopathology and toxicity of THAM are in preparation.

List of Publications:

None

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-9 Metabolism of Cellulose by the Normal
Nonruminant, the Rat

(Study Nos. 3,5 Digestion of Canna Leaf Cellulose;
The Digestion of Tobacco Hemicellulose)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

R. B. Johnson, Ph.D.
and
E. Juknewicz, Sp4

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-9 Metabolism of Cellulose by the Normal Nonruminant, the Rat

Study Nos. 3,5 Digestion of Canna Leaf Cellulose;
The Digestion of Tobacco Hemicellulose

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: R. B. Johnson and E. Juknewicz

RCS MEDDH-288

Unclassified

The digestion of hemicellulose has been studied with the C^{14} technique. Tobacco hemicellulose is digested more rapidly and more completely than is tobacco cellulose. The C^{14} excretion pattern shown by 15 rats: Breath $65.8 \pm 5.3\%$, feces $7.8 \pm 2.0\%$, urine $2.2 \pm 1.3\%$, carcass $11.7 \pm 1.5\%$. Feeding hemicellulose causes the blood pentose level to rise. This is under further investigation. The digestion of a so-called cellulose of the canna plant has been investigated. The C^{14} excretion pattern shown by 5 rats: Breath $57.5 \pm 33.0\%$, feces $27.9 \pm 22.1\%$, urine $2.7 \pm 1.9\%$, carcass $15.6 \pm 2.4\%$. The material appeared to be a hemicellulose rather than a cellulose. However, an amino acid analysis showed the product to be rich in amino acids. The efficacy of an enzyme preparation, Cellase 1000, to cause enhanced cellulose digestion has been investigated in the rat. Although the enzyme rapidly degrades cellulose in certain in vitro systems, it does not cause enhanced cellulose digestion.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-9 Metabolism of Cellulose by the Normal Nonruminant,
the Rat

Study Nos. 3,5 Digestion of Canna Leaf Cellulose;
The Digestion of Tobacco Hemicellulose

Description:

To study the digestion of cellulose and related plant polysaccharides by the nonruminant. The digestibility data would be useful in moving up the animal species series to the human, the terminal animal in the experimental series. The data would also facilitate the prediction of the possibility of making the human G.I. micro-population more cellulolytic.

Progress:

Further studies have shown that tobacco hemicellulose is more completely digested by the rat than is tobacco cellulose. The C^{14} excretion pattern obtained with 15 rats fed tobacco hemicellulose: Breath $65.8 \pm 5.3\%$, feces $7.8 \pm 2.0\%$, urine $2.2 \pm 1.3\%$, carcass $11.7 \pm 1.5\%$. This excretion pattern is in contrast to that seen when tobacco cellulose is fed. The excretion pattern shown by 15 rats when this material was fed: Breath $23.5 \pm 5.1\%$, feces $57.6 \pm 12.1\%$, urine $0.8 \pm 0.5\%$, carcass $5.6 \pm 5.2\%$. A surprising and regular observation was that $C^{14}O_2$ expiration commenced within one hour after feeding hemicellulose, while when tobacco cellulose is fed, such does not occur until the material reaches the cecum (6-8 hours after

Sub-task No. 2-9, Study Nos. 3,5

feeding). Thus, it appears that hemicellulose of the tobacco plant is digested higher in the G.I. tract than is tobacco cellulose.

Feeding 50 mg of hemicellulose also caused the pentose content of the rat's blood to double. This is under further investigation.

The digestion of another cellulose product, a so-called "cellulose" of the canna plant, was studied for comparison. This material gave a C^{14} excretion pattern similar to that seen when tobacco hemicellulose was fed: Breath $65.5 \pm 33.0\%$, feces $27.9 \pm 22.1\%$, urine $2.7 \pm 1.9\%$, carcass $15.6 \pm 2.4\%$. It was thought that the material was a hemicellulose. However, upon submitting the material to amino acid analysis, it proved to have a 10% protein content made up by a fairly complete complement of amino acids. It was decided that it was neither a cellulose nor a hemicellulose, but merely a crude plant material rich in amino acids.

An enzyme preparation, Cellase 1000, which is reputed to have cellulose degrading properties in certain in vitro systems, was obtained for testing from the Wallerstein Laboratories. The material was tested to see if it would cause enhanced cellulose digestion in the rat. The experimental animals were fed cellulose and then Cellase 1000 at various levels. Cellulose digestion was measured as usual. In no case was cellulose digestion enhanced by Cellase 1000. It seems unlikely the enzyme would pass through the stomach and remain in an active state, so it is only reasonable that cellulose digestion would not be enhanced.

Sub-task No. 2-9, Study Nos. 3,5

Summary and Conclusions:

The digestion of hemicellulose has been investigated. Hemicellulose appears to be digested more rapidly and more completely than cellulose. The digestion of a so-called "cellulose" of the canna plant was investigated. It was found not to be a cellulose but some other plant product rich in amino acids. The efficacy of an enzyme preparation, Cellase 1000, to cause enhanced cellulose digestion has been investigated in the rat. Cellase 1000 did not enhance cellulose digestion.

List of Publications:

1. Johnson, R. Bernal and E. Juknewicz. Hemicellulose digestion in the rat. Fed. Proc. 21: 259, 1962.

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-10 The Biochemistry of Calcium and Phosphorus
Metabolism (Study No. 1 The Synthesis of a Radioactive
Vitamin D)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

Z. Z. Ziporin, Ph.D.
and
Jerry Ann Tillotson, B.S.

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-10 The Biochemistry of Calcium and Phosphorus Metabolism

Study No. 1 The Synthesis of a Radioactive Vitamin D

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Z. Z. Ziporin and Jerry Ann Tillotson

RCS MEDDH-288

Unclassified

The procedure for synthesizing a radioactive vitamin D has been worked out. The problem of attaining a very high specific activity has impeded the completion of this project. It has been our aim to achieve a large population of yeast cells low in ergosterol content which would synthesize ergosterol de novo when placed in a suitable medium containing the radioactive tracer. Our findings indicate that cells low in ergosterol are high in squalene and vice versa. New work reported in the literature offers hope that cells low in squalene and low in ergosterol may be grown. Work in this area will be reactivated to determine whether the reported procedures are applicable to our problem.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-10 The Biochemistry of Calcium and Phosphorus Metabolism

Study No. 1 The Synthesis of a Radioactive Vitamin D

Description:

To synthesize a C^{14} -labeled vitamin D with a very high specific activity. With such a compound, we propose to study its metabolism and the mechanisms involved in the calcification of rachitic bone.

Progress:

This phase has been inactive during the past year.

Summary and Conclusions:

The synthesis of a radioactive vitamin D appears feasible. The information which can be obtained by this means, and by no other means at the present time, justifies continuation of the work.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-10 The Biochemistry of Calcium and Phosphorus Metabolism (Study No. 2 Factors Affecting the in vitro Calcification of Rachitic Cartilage)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

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Z. Z. Ziporin, Ph.D.
S. W. Thompson, II, Lt. Col., VC
and
P. P. Waring, B.S.

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-10 The Biochemistry of Calcium and Phosphorus Metabolism

Study No. 2 Factors Affecting the in vitro Calcification of Rachitic Cartilage

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Z. Z. Ziporin, S. W. Thompson, II, and P. P. Waring

RCS MEDDH-288

Unclassified

Beryllium rickets differ from vitamin D-deficiency rickets in that: (1) Bones from beryllium-fed animals do not calcify when vitamin D is administered, except in extremely high doses; (2) the blood phosphorus levels of these animals are extremely low; (3) the bones fail to calcify under in vitro conditions when placed in calcifying solutions. These effects have been attributed to the deficiency of phosphorus leading to a deficiency of hydroxyapatite, the bone salt.

Methods have presently been developed which readily reproduce these effects. These methods will now be employed in an attempt to elucidate biochemical mechanisms involved in beryllium rickets.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-10 The Biochemistry of Calcium and Phosphorus Metabolism

Study No. 2 Factors Affecting the in vitro Calcification of Rachitic Cartilage

Description:

To elucidate mechanisms involved in the calcification of bone.

Progress:

A synthetic diet has been formulated which provides animals with rickets of uniform severity. In testing our methodology, our results concur with published work, namely, that bones from beryllium-fed animals do not calcify in vitro unless they are first immersed in a solution of calcium chloride and then placed in the calcifying solution. The blood phosphorus levels of animals on a low-phosphorus diet are comparable to those of animals on low phosphorus diet to which beryllium has been added. Attempts to define the sites of beryllium deposition in bone by use of the (α, n) reaction, using photographic emulsions for visualization, have not been successful. The materials used to prevent the passage of α -particles attenuate the particles but do not stop them completely. Radioactive beryllium appears to offer a solution to the problem of deposition sites.

Summary and Conclusions:

None at this stage of the investigation.

List of Publications:

None.

FINAL REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

**Sub-task No. 2-26 Thiamine Metabolism (Study No. 2
The Chromatographic Separation of N¹-methylnicotinamide
and Thiamine)**

**U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado**

Chemistry Division

1 July 1961 - 30 June 1962

**Z. Z. Ziporin, Ph.D.
and
P. P. Waring, B.S.**

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-26 Thiamine Metabolism

Study No. 2 The Chromatographic Separation of
N'-methylnicotinamide and Thiamine

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Z. Z. Ziporin and P. P. Waring

RCS MEDDH-288

Unclassified

It has been found possible to remove 85% of the N'-methylnicotinamide from thiamine solutions, and thereby eliminate it as a significant interfering factor in the assay of thiamine. Further study has failed to reveal practical methods for more complete removal of N'-methylnicotinamide, but its complete removal has been found unnecessary for reliable thiamine analyses.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-26 Thiamine Metabolism

Study No. 2 The Chromatographic Separation of
N'-methylnicotinamide and Thiamine

Description:

The separation of N'-methylnicotinamide from thiamine-containing solutions would result in increasing the validity of the chemical assay for thiamine. It was intended that N'-methylnicotinamide may be separated from thiamine either by chromatographic means or by solubility differences in combinations of solvents.

Progress:

By chromatographic means, it has been found possible to separate N'-methylnicotinamide from thiamine to the extent that it does not interfere with the chemical assay for the vitamin. By the use of tracer techniques, it was established that the amount removed was of the order of 85%. Further efforts to improve the degree of separation have not been successful. However, the remaining 15% of N'-methylnicotinamide does not cause significant interference in the assay for thiamine. Therefore, it has not been deemed worthwhile to continue this study and it will be discontinued.

Summary and Conclusions:

Thiamine-containing solutions, such as urine, may now be

Sub-task No. 2-26, Study No. 2

assayed by chemical means without significant interference from N'-methylnicotinamide. This has been accomplished by the chromatographic removal of 85% of the N'-methylnicotinamide.

List of Publications:

None

Sub-task No. 2-26, Study No. 2

assayed by chemical means without significant interference from N'-methylnicotinamide. This has been accomplished by the chromatographic removal of 85% of the N'-methylnicotinamide.

List of Publications:

None

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-26 Thiamine Metabolism (Study No. 3
The Use of the Transketolase Enzyme System as a
Criterion of Thiamine Adequacy in the Chick)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

G. A. Leveille, Ph.D.
H. E. Sauberlich, Ph.D.
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H. J. Schneider, Pfc.

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-26 Thiamine Metabolism

Study No. 3 The Use of the Transketolase Enzyme
System as a Criterion of Thiamine
Adequacy in the Chick

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: G. A. Leveille, H. E. Sauberlich, J. A. Eidelbrock, W. C.
Goad and H. J. Schneider

RCS MENDH-288

Unclassified

An attempt is being made to develop a satisfactory
procedure for hemolyzing avian erythrocytes such that the
red blood cell transketolase activity measurement may be
evaluated as to its sensitivity as an index of thiamine
deficiency in the chick.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-26 Thiamine Metabolism

Study No. 3 The Use of the Transketolase Enzyme
System as a Criterion of Thiamine
Adequacy in the Chick

Description:

A study of biochemical indices of thiamine deficiency in the experimental animal - specifically, in this sub-task, an attempt to adapt the measurement of red blood cell transketolase in chicks.

Progress:

Red cell transketolase activity appears to be a sensitive measure of thiamine adequacy. In attempts to determine the activity of this enzyme in chicken erythrocytes, difficulties were encountered in obtaining a satisfactory hemolysate. When water was added and the cells ruptured by freezing, a gelatinous precipitate formed. The reason for this is not evident, but may very likely be related to the fact that the chick has a nucleated red cell. Work will be carried out in an attempt to find a satisfactory procedure of hemolysing avian erythrocytes such that a homogeneous hemolysate can be obtained.

Summary and Conclusions:

None at this stage of investigation.

List of Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-27 The Biochemical Basis for the Chemotherapeutic
Action of Isoniazid (Study No. 1 Incorporation of Isoniazid
into the DPN of Mycobacterium Tuberculosis)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

Z. Z. Ziporin, Ph.D.
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and
W. C. Morse, Lt. Col., MSC

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-27 The Biochemical Basis for the Chemotherapeutic Action of Isoniazid

Study No. 1 Incorporation of Isoniazid into the DPN of Mycobacterium Tuberculosis

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Z. Z. Ziporin, J. S. Chambers and W. C. Morse

RCS MEDDH-288

Unclassified

While isoniazid has proven to be effective in tuberculosis therapy, nothing is known regarding the mechanisms through which this effect is mediated. From the structural similarity between isonicotinic acid hydrazide and nicotinamide, it would appear possible to incorporate the former compound into DPN in place of nicotinamide, the normal constituent. This would render the DPN ineffective as a coenzyme, and thus interfere in normal intermediary metabolism.

Paper and column chromatography methods have been attempted for isolation of DPN, DPNH, TPN and TPNH from tubercle bacilli without success, nor could the presence of these coenzymes be demonstrated by conventional methods of enzyme assay. Newer methods of handling the cells are being tried to avoid degradation of the nucleotides which must be present in the cells.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-27 The Biochemical Basis for the Chemotherapeutic Action of Isoniazid

Study No. 1 Incorporation of Isoniazid into the DPN of Mycobacterium Tuberculosis

Description:

To determine the biochemical basis for the chemotherapeutic action of isoniazid. This study would investigate the effect of the drug on the Mycobacterium tuberculosis.

Progress:

Conventional methods have been applied for the isolation of DPN, DPNH, TPN and TPNH from the tubercle bacilli. We have not been able to demonstrate the presence of these coenzymes in any of our preparations by the use of paper or column chromatography or by enzyme assays. Since these co-factors must be presumed to be present in these cells, it would appear that the nucleotides are being degraded or otherwise lost by our method of handling. Newer techniques are being applied.

Summary and Conclusions:

None at this stage of the investigation.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions (Study No. 1 The Effect of Variations in the Level of Dietary Phosphorus on Magnesium Balance in the Albino Rat)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

G. E. Bunce, Capt., MSC
P. G. Reeves, 1st Lt., MSC
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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

Study No. 1 The Effect of Variations in the Level
of Dietary Phosphorus on Magnesium
Balance in the Albino Rat

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: G. E. Bunce, P. G. Reeves and T. S. Oba

RCS MEDDH-288

Unclassified

Studies with the weanling male rat have shown that high intakes of inorganic phosphorus will significantly reduce the apparent absorption of magnesium, presumably through the formation of an insoluble complex since fecal phosphorus is also slightly elevated. This effect is not evident, however, when the dietary magnesium is limiting and added principally in an organic combination rather than as the inorganic salt. High levels of inorganic phosphorus promote deposition of calcium in the kidney, but this is opposed by simultaneous elevation of the dietary magnesium. Calcium and magnesium excretion in the urine is enhanced by a low intake of phosphorus.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

Study No. 1 The Effect of Variations in the Level
of Dietary Phosphorus on Magnesium
Balance in the Albino Rat

Description:

The purpose of this study was to determine the effect of variations in the dietary phosphorus:magnesium ratio on the balance of calcium, phosphorus and magnesium in the weanling male albino rat.

Progress:

108 weanling rats were divided into 9 lots and fed either 0.3, 0.5 or 1.0% phosphorus at each of three levels of magnesium; 130, 260 or 1000 ppm. After a 2-week period, total fecal and urine collections were made for 14 days. Analysis of the former revealed that the highest level of phosphorus significantly increased the magnesium content of the feces at the two higher levels of magnesium intake, but not at the lowest level. Similarly, there was a decreased apparent absorption of phosphorus with the highest intake of magnesium when phosphorus was fed at the 1.0% level, but not at the lower levels of phosphorus intake. No effect of either phosphorus or magnesium on fecal calcium was observed at any level of intake.

The lowest level of dietary phosphorus increased the amount of urinary calcium and magnesium by approximately eight-fold and two-fold, respectively. Calcium excretion was also enhanced by the

Sub-task No. 2-28, Study No. 1

highest magnesium intake. Urinary phosphorus, on the other hand, was not affected by the changes in calcium and magnesium, but varied between 1 and 49% of the total phosphorus intake reflecting the extraordinary capacity of the kidney for removal of excess phosphorus.

The kidneys were analyzed at the end of the experiment for total calcium, phosphorus and magnesium. In agreement with reports by other labs, no loss of magnesium from the kidney could be demonstrated chemically. Accumulation of both calcium and phosphorus in the kidney was associated with either a low intake of magnesium or a high intake of phosphorus. A more than additive deposition occurred with combinations of low magnesium and high phosphorus. This accumulation appeared upon histopathologic examination to begin as urolithiasis. Nephrocalcinosis was also present; however, in the more severe cases.

Summary and Conclusions:

The interaction of phosphorus and magnesium on the apparent absorption of both of these elements may be explained by postulating the formation of a highly insoluble complex when both are present in the gastrointestinal tract principally as the inorganic salts. An alternative explanation, however, could be an increase in the excretion of one via the fecal route when the other is present in excess, an effect which might be limited by the amount of available body stores. The use of radioactive isotopes to answer this question is contemplated if sufficient time and personnel are available.

Sub-task No. 2-28, Study No. 1

The results of this experiment conclusively demonstrate that a high intake of phosphorus promotes pathological deposition of calcium in the collecting tubules of the rat kidney while at the same time markedly reducing calciuria. It is also evident that magnesium retards this deposition. An understanding of the mechanisms involved in these changes would be of great value in the study of the etiology of human kidney stone disease.

List of Publications:

1. Bunce, G. E., P. G. Reeves, T. S. Oba and H. E. Sauberlich.
The effect of dietary phosphorus and magnesium on calcium, phosphorus and magnesium balance and kidney calcium deposition in the rat.
(In preparation)

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions (Study No. 2 Carbohydrate Metabolism in the Zinc Deficient Rat)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

G. E. Bunce, Capt., MSC
P. G. Reeves, 1st Lt., MSC
and
T. S. Oba, Sp5

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 2 Carbohydrate Metabolism in the
Zinc Deficient Rat)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: G. E. Bunce, P. G. Reeves, and T. S. Oba

RCS MEDDH-288

Unclassified

Since the metabolic defects responsible for the lesions of zinc deficiency are largely unknown, this study was initiated to investigate the relationship of zinc to carbohydrate metabolism in general, with particular emphasis on the possible interactions with thiamine.

Although no effect of a marginal zinc intake on the expiration of $C^{14}O_2$ could be detected following administration of C^{14} labeled glucose, it did appear that synthesis of the B_1 dependent enzyme transketolase was reduced. This possibility will be studied further.

No evidence was found of a sparing effect of thiamine on zinc deficiency nor of zinc on thiamine deficiency.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 2 Carbohydrate Metabolism in the Zinc Deficient Rat)

Description:

The purpose of this study is to investigate the carbohydrate metabolism of the zinc deficient rat as a means of determining more specifically the role and requirement of this mineral. Specific attention will be paid to a postulated interaction between zinc and thiamine.

Progress:

Diets low in either zinc or thiamine or both were fed to weanling male rats for six weeks at which time total weight was significantly depressed in the low zinc and low thiamine groups as compared to the supplemented control lot. The dually deficient animals showed depressed weight gains approximately equal to the sum of the depressions noted in the singly deficient lots. No sparing effect of either nutrient upon the other was noted.

No difference in rate or total excretion of $C^{14}O_2$ was found following administration of C^{14} labeled glucose. Red cell transketolase activity was reduced in all groups which received low

Sub-task 2-28, Study No. 2

intakes of B₁. Restoration of enzyme activity following addition of thiamine pyrophosphate to the reaction tubes was not optimal, however, when the zinc intake was marginal indicating a possible deficiency of enzyme.

Studies of the effect of low zinc intake on the capacity of liver homogenates to oxidize pyruvate are planned but have not yet been completed.

Summary and Conclusions:

The possibility that transketolase synthesis is reduced with a low zinc intake deserves further attention particularly in view of the fact that the red cell transketolase assay is being studied as an indicator of B₁ adequacy in human populations in Southeast Asia, an area where zinc intake has been reported to be low.

List of Publications:

None

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions (Study No. 3 The Effect of the Level of Dietary Protein on the Magnesium Requirement)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

13 July 1961 - 30 June 1962

G. E. Bunce, Capt., MSC
P. G. Reeves, 1st Lt., MSC
and
T. S. Oba, Sp5

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 3 The Effect of the Level of Dietary Protein on the Magnesium Requirement)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

13 July 1961 - 30 June 1962

Authors: G. E. Bunce, P. G. Reeves, and T. S. Oba

RCS MEDDM-288

Unclassified

Dietary studies with both the chick and the rat have indicated an increased requirement for magnesium in these species following elevation of the dietary protein. Balance studies in the rat, however, did not show a decreased retention of this mineral as a consequence of the higher intakes of protein. It was therefore concluded that the increased requirement reflected a vital though perhaps indirect role of magnesium in protein metabolism. Support of this contention was provided by the finding that total serum protein was depressed and certain urinary amino acids were elevated when the magnesium level was adequate. The concentration of magnesium in the liver, a vital organ for protein metabolism, was also found to be greatly diminished in the rats fed the lower magnesium intakes.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

(Study No. 3 The Effect of the Level of Dietary Protein on the Magnesium Requirement)

Description:

The purpose of this study is to investigate the postulated relationship between the level of dietary protein and the magnesium requirement. Particular attention is to be paid to the role of magnesium in protein and amino acid metabolism.

Progress:

In the first experiment, it was shown that the mortality rate of chicks fed 100 ppm of magnesium was doubled by elevation of the dietary protein from 24 to 36%. At a level of 600 ppm, there was no difference in the weight gain or appearance of the groups fed either 24 or 36% protein. When the dietary protein was lowered to 12%, growth of the chicks fed either 100 or 600 ppm was equal, but inferior to that obtained at the highest intake of magnesium and 24 or 36% protein.

In a second experiment, male weanling rats were fed either 12 or 36% protein at three levels of magnesium: 100, 200 or 600 ppm. At the 100 ppm level, weight gain, although subnormal in both groups, was significantly reduced in the rats fed the higher level

Sub-task 2-28, Study No. 3

of protein and the rate of appearance of the hyperemia characteristic of magnesium deficiency was greatly accelerated in this group. Weight gain was significantly better in the high protein fed lots at either 200 or 600 ppm magnesium but there was no difference in gain between the two mineral concentrations at a given protein level. Hyperemia was evident in 6 of 8 animals in the 200 ppm magnesium, 36% protein lot although this amount is generally considered adequate at lower levels of dietary protein. Balance studies did not reveal any statistical reduction in magnesium apparent absorption or retention as the dietary protein was increased at any given level of magnesium fed.

A role of magnesium in protein metabolism seemed to be suggested by these results. This was supported by the finding that serum protein values were lower in the rats fed the lowest level of magnesium and the highest level of protein and in the observation of a 50% reduction in the urinary excretion of certain amino acids in the rats consuming 12% protein and 600 ppm magnesium as opposed to the values found with 12% protein and lower intakes of magnesium.

The importance of the liver in protein metabolism prompted the determination of the magnesium content of this organ. The average magnesium content of the livers of the rats fed the two lower levels of this mineral and either level of protein were found to be significantly depressed by approximately 50 and 25% respectively from the values found at 600 ppm of magnesium. The metabolic significance of

Sub-task 2-28 No. 3

this reduced level of tissue magnesium will be investigated in subsequent experiments.

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Summary and Conclusions:

It seems reasonable to conclude that an increase in dietary protein elevates the requirement for magnesium. Since balance studies do not give evidence of decreased retention of magnesium when the protein is elevated, a vital role of this mineral in protein and amino acid metabolism is indicated. Results of determinations of total serum proteins and the urinary excretion of amino acids support this thesis. The magnesium content of the liver has been shown to be significantly depressed under conditions where magnesium intake was slightly submarginal or marginal.

List of Publications:

1. Bunce, G. E., P. G. Reeves, T. S. Oba and H. E. Sauberlich.
Dietary protein and the magnesium requirement (in preparation).

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03

Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions (Study No. 6 The Effect of Dietary Copper, Molybdenum, and Zinc on the Distribution of Zn-65 in the Mammalian System)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

5 February 1962 - 30 June 1962

Kenneth E. Kinnamon, Capt., VC
and
George C. Bunce, Capt., MSC

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

Study No. 6 The Effect of Dietary Copper, Molybdenum, and Zinc on the Distribution of Zn-65 in the Mammalian System

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

5 February 1962 - 30 June 1962

Authors: Kenneth E. Kinnamon and George C. Bunce

RCS MEDDH-288

Unclassified

Zinc has been found to be present in biological material in amounts considerably greater than any of the other trace elements, yet information concerning its metabolism is meager. The relatively recent discovery of zinc in several highly purified enzymes has revealed the diversity of its role in metabolism. This wide body distribution in many cell types suggests its potential importance in mammalian biochemical pathology.

The interrelationship of zinc with other trace elements, initially molybdenum and copper, will be studied by observing the distribution of an intraperitoneal injection of radioactive zinc (Zn-65) in

2-28, Abstract, Cont'd

rats which have been fed diets containing varying levels of these three trace elements. The animals will then be sacrificed and the distribution and quantities of both total and radioactive zinc in various tissues will be analyzed.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-28 Studies on Mineral Metabolism and Interactions

Study No. 6 The Effect of Dietary Copper, Molybdenum, and Zinc on the Distribution of Zn-65 in the Mammalian System

Description:

The distribution of zinc is important as a possible etiological agent in metabolic disease and it is possible that its deviation of location (e.g. urine level, blood level) may be used as a diagnostic tool to ascertain other specific disease entities. It is known that utilization of a given mineral nutrient may be profoundly influenced, even to a disease state, by other mineral constituents in the diet. Furthermore, a knowledge of the interrelationships among various nutrients in a diet is essential for an understanding of their quantitative requirements for the body.

This study is designed to observe the interrelationships among three trace metals; copper, molybdenum, and zinc, and to note how these different elements and their combinations affect the distribution and excretion of radioactive zinc in the mammalian system. Such studies are basic to an understanding of the mechanism of zinc utilization, may demonstrate a specific dietary zinc antagonist, and possibly give a clue to metabolic abnormalities which at the present time have an unknown etiology.

2-28

Progress:

Rats are presently being fed the respective experimental diets in which there are varying levels of copper, molybdenum, and zinc. Zn-65 injections have not yet been made to ascertain the distribution in these animals under the various dietary conditions, but are planned.

Conclusions:

None at this stage of the investigation.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 - Biochemistry

Sub-task No. 2-29 Pyridoxine Metabolism (Study Nos.
1,2,3 The Influence of Pyridoxine Deficiency
on Serum and Tissue Enzymes)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Chemistry Division

1 July 1961 - 30 June 1962

G. A. Leveille, Ph.D.
H. E. Sauberlich, Ph.D.
J. A. Edelman, Sp4
W. C. Goad, Sp4
and
E. J. Schneider, Pfc

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ABSTRACT

Project No. 62-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-29 Pyridoxine Metabolism

(Study Nos. 1, 2, 3 The Influence of Pyridoxine
Deficiency on Serum and Tissue Enzymes)

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons
General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: G. A. Leveille, E. E. Sauberlich, J. A. Edelbrock, W. C.
Goad, and E. J. Schneider

RCS MEDDH-288

Unclassified

The activity of serum glutamic-oxaloacetic and
glutamic pyruvic transaminases was found to be diminished
in rats and chicks fed a pyridoxine deficient diet. How-
ever, the addition of sub-optimal levels of dietary
pyridoxine, as judged by body weight gains, did not show
any depression in the activity of either enzyme. It is
felt that these enzymes lack the sensitivity required
for their employment as a criterion of the pyridoxine
status of rats and chicks.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 03 Biochemistry

Sub-task No. 2-29 Pyridoxine Metabolism

(Study Nos. 1,2,3 The Influence of Pyridoxine
Deficiency on Serum and Tissue Enzymes)

Description:

A study of transaminase enzyme assays to assess their sensitivity and usefulness as a criterion of the pyridoxine status of experimental animals.

Progress:

The activity of two serum transaminases, glutamic-oxaloacetic and glutamic pyruvic transaminases, was determined in chicks and rats fed diets containing varying levels of pyridoxine. A difference could be detected in the enzyme activity of animals fed a pyridoxine deficient or supplemented diet. However, no differences were observed in the transaminase activity of animals fed different levels of pyridoxine.

It is felt that the methods employed for this study were not sensitive enough to allow for accurate determination of the differences which may have existed between the groups of animals given the varying levels of pyridoxine. Other procedures will be studied to determine the feasibility of employing serum transaminase activity as a criterion of pyridoxine status of individuals.

Sub-task No. 2-29, Study Nos. 1,2,3

Summary and Conclusions:

Serum glutamic-oxaloacetic and glutamic-pyruvic transaminases have been shown to lack sensitivity as a criterion of pyridoxine status of experimental animals.

Publications:

None at this state of the investigation.

FINAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 09

Physiology

Sub-task No. 5-9 Exchange Rate of Doubly Labeled Glycine
(Study No. 1 Instrumentation and Method Development)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

William S. Worth, M.S.
with technical advice of
B. M. Tolbert, Ph.D.*

*Chemistry Department, University of Colorado, Boulder, Colorado

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-9 Exchange Rate of Doubly Labeled Glycine

Study No. 1 Instrumentation and Method
Development

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: William S. Worth with technical advice of
B. M. Tolbert

RCS MEDDH-288

Unclassified

The development of instrumentation and procedures for continuous analysis of expired gas from an animal which has been given a tracer dose of labeled amino acid is complete. These techniques are now being used to obtain information regarding the metabolism of Glycine-1-C¹⁴ and Glycine-2-C¹⁴ in dogs, as well as other nutrients and metabolites.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-9 Exchange Rate of Doubly Labeled Glycine

Study No. 1 Instrumentation and Method
Development

Description:

Instrumentation and methods of analysis have been developed to determine the in vivo rate of decarboxylation of amino acids in normal dogs whose metabolic pathways have been altered by various experimental procedures.

Progress:

Instrumentation and techniques for continuously recording the evolution of $C^{14}O_2$ from a conscious dog have been developed. A "Radioactive-Gas Analyzer" was used. This instrument incorporates a vibrating reed electrometer ionization chamber, a CO_2 analyzer, a dry gas meter, and a six channel recorder. A dog mask was fabricated for the collection of expired air from the conscious dog. To insure complete intravenous infusion of the administered labeled compounds a semi-permanent Silastic catheter was placed in the dog's jugular. With the recorded data obtained by these methods we are now able to evaluate the oxidative metabolism of labeled compounds in vivo and observe changes in both transient phases and the steady state utilization in various physiologic and pathologic conditions.

Summary and Conclusions:

These techniques have been successfully used in experiments using C^{14} labeled glycine, glucose, lysine, vitamins K and C, and mevalonic acid in rats, dogs, and humans. Results from these experiments have been or will be reported under the particular biological studies for which the instrumentation and methods are used. Major instrumentation research and development has been completed and this phase of the study is now terminated.

List of Publications:

1. Nims, Robert M., and Worth, William S. Fabrication of a Canine Respiratory Head Mask. J. Applied Physiol. 16: 1139, Nov. 1961.
2. Worth, William S., and Tolbert, Burt M. Method of Calculating Data from the "Radioactive Gas Analyzer", Report to be written.

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 09

Physiology

Sub-task No. 5-9 Exchange Rate of Doubly Labeled Glycine
(Study No. 2 The Relation of Glycine Metabolism to Glucose
Utilization)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 December 1961 - 30 June 1962

William S. Worth, M.S.
George C. Schussler, Capt., MC
Robert M. Nims, Lt. Col., VC

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-2 Exchange Rate of Doubly Labeled Glycine

Study No. 2 The Relation of Glycine Metabolism
to Glucose Utilization

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 December 1961 - 30 June 1962

Authors: William S. Worth, George C. Schussler and Robert M. Nims

RCS MEDDH-288

Unclassified

The metabolism of glycine-1-C¹⁴ in pancreatectomized animals was studied. Animals maintained on insulin converted a significantly higher percentage of administered glycine-1-C¹⁴ to C¹⁴O₂.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-9 Exchange Rate of Doubly Labeled Glycine

Study No. 2 The Relation of Glycine Metabolism
to Glucose Utilization

Description:

Excessive loss of protein because of decreased anabolism and increased catabolism is a major deleterious effect of diabetes mellitus. It is apparent that glucose metabolism is intimately associated with the metabolism of protein. These studies were designed to investigate the effect of glucose metabolism on the catabolism of amino acids.

Progress:

The radioactive gas analyzer previously described was employed to measure the production of $C^{14}O_2$ from glycine- $l-C^{14}$ in surgically pancreatectomized dogs. Glycine- $l-C^{14}$ was administered rapidly as a single intravenous dose in five to ten microcurie quantities. The rate of $C^{14}O_2$ evolution in the expired air was maximal in approximately twenty minutes. In animals receiving insulin an average of 18% of the injected radioactivity was recovered as $C^{14}O_2$ within the first hour. When insulin was withheld (usually for three days prior to the experiment) an average of only 11% of the label was recovered as $C^{14}O_2$.

5-9

Summary and Conclusions:

These studies appear to demonstrate a decreased oxidative metabolism of glycine when glucose utilization is decreased in the diabetic dog.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 09

Physiology

Sub-task No. 5-24 Functional Aspects of Body Composition

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

Thomas H. Allen, Ph.D.
and
Harry J. Krzywicki, M.S.
Kenneth S. K. Chinn, B.S.
Robert M. Nims, Lt. Col., VC
Robert W. Allard, SP4

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-24 Functional Aspects of Body Composition

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Thomas H. Allen, Harry J. Krzywicki, Kenneth S.K. Chinn,
Robert M. Nims and Robert W. Allard

RCS MEDDH-288

Unclassified

Methods previously developed in this laboratory have been utilized to extend body volume measurements to additional subjects and to relate these measurements to other physiological observations. Additional data is needed to statistically validate the results. A small sized volumeter to accommodate children approximately one meter tall has been constructed. Diurnal production of abdominal gases was investigated in the water displacement volumeter in an attempt to derive predicting equations for estimating intestinal gas volumes.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-24 Functional Aspects of Body Composition

Description:

Body composition studies are essential in a variety of disciplines. Methodology is complex. A simple device, the water displacement body volumeter, developed and in use at this laboratory, has been used effectively under a variety of conditions. Body fat and fat free body weight can easily be computed from measurement of total body tissue volume. During the past year efforts have been made to extend observations to a more varied population, to obtain data in support of other projects currently in progress, and to relate volumeter data to other physiological parameters. Further refinement of technique required a study of the contributions of abdominal gas to body volume.

Progress:

Body volumes and weights of twenty men over 50 years of age were made during the past year, adding to the accumulated data on relation of body composition with age. Approximately seven 50 to 60 year olds and thirteen 60 to 70 year olds, plus whatever number beyond 70 years of age might be available are still needed to make the data statistically reliable.

5-24, Progress, Cont'd

Of the boys 8 to 14 years of age previously measured it was possible to re-measure six individuals semi-annually, and of the adult females proposed for study by Major Mary Tkacik, ANC, a total of fifteen women were finally measured in the body volumeter. Eight girls ranging from 7 to 14 years of age were also measured by Major Tkacik. Future work will include accumulation of data on older men, while data on more children and women is most desirable before a final report is to be submitted.

The water displacement body volumeter was used to measure volume of the metabolic ward subjects to gather ancillary data in two studies done primarily for other purposes. One was designed to test the digestibility of microcrystalline cellulose and its effect upon digestibility of other macronutrients. The second tested the effect of periodicity of eating on intermediary metabolism. In both instances 24 hour urinary creatinine was determined daily in an effort to further refine the estimation of the lean body mass of these subjects. In the latter study heavy water was also administered to estimate total body water. In addition, sweat is to be collected and tested for creatinine and deuterium losses, if any, on the day of deuterium administration. Collection and calculation of data is still in progress at the time of this writing.

5-24, Progress, Cont'd

The plastic chamber following body conformation previously built in order to permit measurement of body volume by gas dilution (thus avoiding immersion under water which is not applicable to sick patients) has received further study. However, efforts to measure accurately the volume of the empty plastic chamber using argon have failed due to the inability of the mass spectrometer to repeatedly analyze for argon with less than 1.0% error. The system is not to be discarded since the plastic chamber is smaller than existing chambers and therefore potentially more accurate, and it is also designed to accomodate sick patients with minimal inconvenience. Instrumentation and methods for achieving the desired accuracy with the gas dilution volumeter are currently being evaluated. Diurnal variations in abdominal gas contributions to body volume were measured in several subjects. The effects of the ingestion of food and liquids and of flatus were noted. Mathematical calculation of these results is still under way. It is anticipated that equations for the prediction of abdominal gas will be derived.

Summary and Conclusions:

Dynamic aspects of body composition are being developed and methods are being perfected which make it easy to quantify body fat burden, size of the fat-free body, assessment of edema through partial body volume measurements, physical

5-24, Summary and Conclusions, Cont'd

proficiency, and other interrelated indices, such as 24 hour urinary creatinine and body water measurements by isotope dilution.

List of Publications:

None at this state of the investigation. Some additional subjects need to be studied before conclusions and a final report can be made.

FINAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 09

Physiology

Sub-task No. 5-25 Hypertrophic Pulmonary Osteoarthropathy

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

Robert M. Nims, Lt. Col., VC
and
Kaye H. Kilburn, M.D.

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-25 Hypertrophic Pulmonary Osteoarthropathy

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: Robert M. Nims and Kaye H. Kilburn

RCS MEDDH-288

Unclassified

A total of thirty-two dogs were operated in an attempt to produce either a right to left central vascular shunt (29 animals) or a peripheral arterio-venous fistulae (3 animals). This was done as a preliminary stage to investigate the pathogenesis and pathophysiology of hypertrophic pulmonary osteoarthropathy. In 29 dogs with central vascular shunts, only 4 were patent for significant periods of time (7 to 21 months). These 4 successfully produced shunts (of 30-40%) were all in the group of 15 dogs in which 3/4" internal diameter crimped Teflon vascular prosthesis was used between the pulmonary artery and the left atrium. Although arterial oxygen saturation was significantly reduced there was no success in producing osteoarthropathy. One of the 2 dogs with persistent arterio-venous fistulae showed

5-25, Abstract (Cont'd)

a thickened bone cortex radiographically and one small area of periosteal elevation. These changes were not visible grossly at necropsy.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-25 Hypertrophic Pulmonary Osteoarthropathy

Description:

An investigation of the pathogenesis and pathophysiology of hypertrophic pulmonary osteoarthropathy by producing a large right to left central vascular shunt (main pulmonary artery to left atrium). This will attempt to duplicate a classic experiment by Mendlowitz and Lester in 1941. They demonstrated periosteal elevation with patchy calcifications on the radius and ulna of one dog eight months after producing a central shunt of 49%.

Progress:

Various methods of producing right to left central vascular shunts were investigated. In a series of nine animals, the left auricular appendage was anastomosed to the main pulmonary artery. None of these shunts remained patent.

In another series, using five dogs, an attempt was made to anastomose the inferior vena cava to the left atrium. None of the animals survived the surgery.

With fifteen dogs a crimped Teflon vascular prosthesis, 3/4" internal diameter, was used to produce a shunt from the main pulmonary artery to the left atrium. This procedure produced the only successful shunts. However, only four of the dogs maintained a patent shunt for any significant period of time (seven, thirteen, thirteen and twenty-one months).

5-25, Progress (Cont'd)

A series of three dogs had arterio-venous shunts between the femoral artery and vein, high on the left hind leg. Two of these had patent shunts for periods of twenty-seven months. The other shunt closed shortly after surgery.

These animals were followed by monthly x-rays of the chest and extremities, as well as periodic arterial oxygen saturation determinations. If indicated, the shunts were tested for patency by performing right heart angiography plus a modified dye dilution cardiac output study. If negative by these tests, a thoracotomy was performed to directly observe the shunt.

Summary and Conclusions:

In twenty-nine dogs with central vascular shunts, only four were patent for significant periods of time. None of these demonstrated roentgenographic or gross necropsy evidence of periosteal elevation. Two animals of three had persistent arterio-venous fistulae. One of these had a thickened cortex and roentgenographically had one small area of periosteal proliferation. This could not be detected grossly at necropsy. The other showed no changes.

The attempt to produce inferior vena cava to left atrium shunts was a failure. Of five dogs, none survived the surgery.

In our experience, four dogs with central vascular shunts estimated at 30-40% did not develop lesions associated with hypertrophic pulmonary osteoarthropathy although the shunts

5-25, Summary and Conclusions (Cont'd)

were functional for periods of seven, thirteen, thirteen and twenty-one months.

Central right to left vascular shunts which significantly lowered the arterial oxygen saturation were not successful in producing experimental hypertrophic pulmonary osteoarthropathy.

List of Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 09

Physiology

Sub-task No. 5-27 Thyroid Iodine Metabolism
(Study No. 1 Regulation of Thyroid Hormone
Synthesis)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

George C. Schussler, Capt., MC
and
William S. Worth, M.S.
Tillman L. Eddy, SP5
Joseph L. Baber, SP4

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-27 Thyroid Iodine Metabolism

Study No. 1 Regulation of Thyroid Hormone
Synthesis

U. S. Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: George C. Schussler, William S. Worth, Tillman L. Eddy
and Joseph L. Baber

RCS MEDDH-288

Unclassified

A radiosensitive endogenous inhibitor of iodination
has been demonstrated in sheep thyroid homogenates. It
has not been possible to demonstrate an effect of this
inhibitor in vivo.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-27 Thyroid Iodine Metabolism

Study No. 1 Regulation of Thyroid Hormone
Synthesis

Description:

A dialyzable endogenous inhibitor of the formation of moniodotyrosine in sheep thyroid homogenates has been shown to be radiosensitive. This finding explains a marked sensitivity to radiation in iodination experiments in sub-cellular thyroid preparations. See last year's Annual Progress Report.

Progress:

Attempts were made to extend these findings to the effect of I^{131} on total iodide uptake in rats. No significant relation between uptake and dose was found.

Summary and Conclusions:

A radiosensitive inhibitor of iodination in the thyroid was demonstrated in vitro but not in vivo. Data is currently being prepared for publication.

5-27

List of Publications:

1. Schussler, George C. "Effect of Varying Concentrations of I^{131} on Incorporation of Iodide Into Protein by Sheep Thyroid Homogenates." Abstract presented at 45th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 10-14, 1961

ANNUAL PROGRESS REPORT

Project No. 6X99-26-001

BASIC RESEARCH IN LIFE SCIENCES

Task No. 09

Physiology

Sub-task No. 5-27 Thyroid Iodine Metabolism
(Study No. 2 An In Vitro Thyroxine Deiodinating
System)

U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

Physiology Division

1 July 1961 - 30 June 1962

George C. Schussler, Capt., MC
and
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Dale E. Fuller, PFC

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ABSTRACT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-27 Thyroid Iodine Metabolism

Study No. 2 An In Vitro Thyroxine Deiodinating System

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver 30, Colorado

1 July 1961 - 30 June 1962

Authors: George C. Schussler, Tillman L. Eddy, and Dale E. Fuller

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Unclassified

The properties of an in vitro deiodinating system have been studied. In a liver homogenate maximal efficiency was obtained at high dilution. The system proved sensitive to stimulation by flavin adenine dinucleotide and possibly triphosphopyridine nucleotide. It was inhibited by very low concentrations of certain thyroxine analogues and human serum. Methimazole was inhibitory at higher concentrations.

BODY OF REPORT

Project No. 6X99-26-001 BASIC RESEARCH IN LIFE SCIENCES

Task No. 09 Physiology

Sub-task No. 5-27 Thyroid Iodine Metabolism

Study No. 2 An In Vitro Thyroxine Deiodinating System

Description:

The degradation of thyroxine is altered to a greater extent than is the protein bound iodide in myxedema and thyrotoxicosis. Conversely, a normal daily thyroxine degradation may be associated with abnormal levels of plasma thyroxine binding proteins in eumetabolic patients despite elevated or depressed serum protein bound iodide. It appears, therefore, that the degradation of thyroxine is more closely related to hormone action than is the absolute concentration in the blood. Although it is apparent that thyroxine degradation must occur in the tissues, the lack of information regarding this deiodinating system has limited our concept of the regulation of thyroxine degradation to the factors measured in serum samples, concentration of the hormone and thyroxine binding capacity of the serum protein. The current studies have been undertaken to investigate the characteristics of the tissue thyroxine degrading system.

Progress:

A rat liver homogenate in pH 7.4 tris buffer was utilized. The substrate was $3'5'I^{131}$ labelled thyroxine. The system was incubated at $37^{\circ}C$ for one hour. The radioactive products were analyzed by paper chromatography and scanned by an integrating chromatogram scanner. In agreement with results obtained by other investigators the products of incubation were I^{131} as iodide and an unidentified substance that remained

at the origin of the chromatographic strips. No other I^{131} labelled compounds were obtained, even in highly active homogenates. Increasing dilution of the homogenate not only increased the efficiency of the system but increased total deiodination. The homogenate could also be stimulated by heating to 100° C for 10 minutes and by overnight storage at 4° C. Dialysis and prolonged incubation at 37° C were inhibitory.

The addition of flavin adenine dinucleotide was consistently stimulatory in the fresh homogenate and triphosphopyridine nucleotide seems to have a synergistic action. However, the stimulation achieved by these coenzymes was considerably less than that obtained by heating or storage.

Percentile deiodination was readily inhibited by increasing the concentration of thyroxine. The addition of non-radioactive triiodothyronine was equally effective in decreasing the percentile deiodination of thyroxine. Two dimensional chromatography of homogenates incubated with inhibitory concentrations of triiodothyronine did not reveal the accumulation of radioactive triiodothyronine, suggesting that this is not an intermediate compound in the deiodination of thyroxine. Other iodinated amino acids have been tested for their ability to inhibit thyroxine deiodination. The iodinated tyrosines are without effect. A number of tri- and tetraiodinated thyronine derivatives have been inhibitory at low molar ratios to substrate thyroxine. Methimazole was an effective inhibitor of deiodination. This effect occurred at considerably higher concentrations than those at which the thyroxine analogues were effective.

As has been previously demonstrated by others, the addition of human serum is capable of inhibiting deiodination. The homogenate

described here is extremely sensitive to such inhibition. A 1/2000 dilution of serum effectively inhibits deiodination. This is apparent at concentrations of thyroxine greatly in excess of those required to saturate the specific serum thyroxine binding proteins (thyroxine binding globulin and prealbumin). A similar effect can be demonstrated with serum albumin alone.

Summary:

A liver homogenate has been studied as a model of the in vivo thyroxine deiodinating system. It appears to be heat stable and sensitive to stimulation by flavin adenine dinucleotide and possibly by triphosphopyridine nucleotide. It is readily inhibited by human serum in small quantities and this inhibition is probably due to serum albumin. Methimazole is also an effective inhibitor of the deiodinating system. A variety of thyroxine analogues are effective inhibitors of the deiodination of thyroxine.

List of Publications:

1. Schussler, George C. "An In Vitro Thyroxine Deiodinating System", abstract: Clinical Research 10:235 (1962)

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